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A STUDY OF DELAYED GERMINATION IN ECONOMIC SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 204

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(WITH ONE FIGURE)

This paper presents the results of an attempt to discover some of the practical problems that seedsmen and growers have to meet, and to work out, so far as possible, practical methods of solving these problems. The seeds tested were furnished by six of the leading seed houses of the United States.

In the present state of our knowledge it can be said that delayed germination and poor germination are due to one or more of the following causes: hard-coatedness, the need of after-ripening, exclusion of oxygen by the seed coat, the effect of frost on seeds, fungi on or in seeds, and of course the presence of seeds containing dead embryos.

Hard-coatedness

The condition of hard-coatedness in the seeds of legumes is well known. To overcome this condition investigators have used hot water, chemicals, and mechanical devices for scratching or puncturing the seed coat.

The use of hot water for forcing germination is undoubtedly older than the references to it in periodical literature. It was recommended by BRUYNING (**6**) in 1893 for seeds of *Ulex europaeus*,

and by WERNICKE (**30**) in 1895 for several different kinds of seeds. Mention may also be made of the work of JARZYMOWSKI (**17**) in 1905 with seeds of various economic legumes, and of BOLLEY (**3**) in 1912 with those of alfalfa. BOLLEY obtained positive improvement in germination if exposure to a given temperature was not long enough to kill the embryo.

Treatment with concentrated sulphuric acid dates from the work of ROSTRUP (**25**) in 1896–1897. It was also used by TODARO (**29**) in 1901, by HILTNER (**14**) in 1902, by JARZYMOWSKI (**17**) in 1905, and by BOLLEY (**3**), and LOVE and LEIGHTY (**22**) eight years later. Increased germination was obtained in all these cases.

Treatment with other chemicals has included the use of ether, ethyl, and other alcohols (VERSCHAFFELT **31**, 1912), chloroform, sodium hydroxide, postassium hydroxide, potassium nitrate, and mercuric chloride. Of these, the lower alcohols are the only ones that are very effective.

There are obvious practical objections, however, to the use of either hot water or chemicals. As a consequence, there have been numerous efforts to devise means for the mechanical treatment of hard-coated seeds. In Germany, KUNTZE and HUSS (**16**), working about 1890, were able with a scratching machine to increase the germination of *Lathyrus sylvestris* 83 per cent, *Vicia Cracca* 71 per cent, and *Astragallus Glycyphyllos* 77 per cent.

Somewhat later MICHALOWSKI devised an apparatus in which the seeds were passed between two rollers, one of rubber, the other of rough steel. Smaller sorts of seeds were badly crushed by such a mechanism, and it was later displaced by two others, one designed by the Wissinger Seed Co., of Berlin, the other, called a "preparator," by H. NILSSON of the experiment station at Svalöf, Sweden. In both of these the seeds are thrown from a revolving disk against the concave surface of a circular rough stone, within which the disk revolves. HUME and GARVER (**15**), using the "preparator," obtained a definite increase in the germination of seeds of *Medicago sativa*, *M. media*, and *M. falcata*. Another machine now in use in England has made it possible, according to CARRUTHERS (**7**), the designer, to buy clover seed guaranteed to germinate 98–100 per cent. The seeds to be treated are fed into a revolving cylinder

lined with sharp, close-set steel points against which the seeds are thrown and scratched as the cylinder revolves.

Mention should be made here also of an apparatus invented by KÜHLE (**19**) for scraping the rough outer covering from sugar beet "seed." Very satisfactory results have been obtained by its use, since "seeds" so treated absorb water better than untreated ones, and germinate more rapidly; they also give a better total germination, on account of the removal of fungus-infected material from the outside of the "seed," especially if this removal is followed by treatment with some fungicide.

With any one of the machines here described except the last, which serves a slightly different purpose, it has been found difficult to treat every seed that passes through and, at the same time, to avoid serious cracking of the coat or bruising of the entire seed (GLOCKENTOEGER **11**).

It is believed that these difficulties have been avoided in a machine devised and in use during the winter of 1912–1913 at the Hull Botanical Laboratory of the University of Chicago. This machine consists of a direct pressure blower, furnished by the Connersville Blower Co., to which is attached an apparatus through which seeds can be fed and blown against the points of a bank of needles. In experiments conducted with this machine, the blower was driven by a two horse-power motor and gave pressures as high as 2.5 pounds to the square inch. The needles used were of three sizes, nos. 4 and 11 sewing needles and no. 4 darning needles, made up into three different cylindrical bunches or banks, each bank of course consisting of only one size of needles. The needles were held together by solder at the eye end and by wire or a ferrule one-half to two-thirds of the distance from the eye to the point.

In the cut here shown (fig. 1) the needles are about half an inch from the end of the air tube. In practice a screen cap is placed over the needles and the tube as a covering for a glass jar beneath, into which the seeds fall. To use the apparatus, valve *e* is closed and valve *b* is opened; seeds are poured into compartment *c;* valve *b* is closed and the blower started; valve *e* is then opened wide enough to let the seeds out, but not so wide that they interfere with each other as they strike the needle points. It is

plain that the distance the valve is to be opened will vary with different seeds, but will not be at any time particularly hard to determine. With valve *b* closed, there was no difficulty in getting the seeds down into the air tube; with it open they would be blown out at *d*.

The opening at *h* is five-eighths of an inch in diameter and will accommodate seeds of sweet peas, lupines, *Lathyrus*, honey locust,

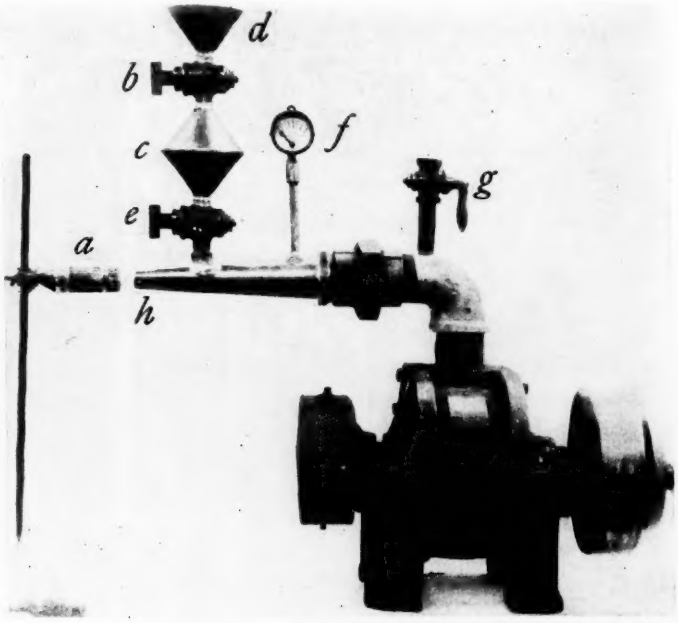

FIG. 1.—Apparatus for increasing the germination of hard-coated seeds by blowing them against a bank of needle points.

etc. When smaller seeds are to be treated, an attachment with three-eighths inch feed, valves, and air tube can be adjusted readily. Valve *g* can be used to regulate the pressure, as read on the pressure gauge *f*.

Tests were made of treated and untreated seeds, on filter paper kept moist with distilled water, at a temperature of 23–25° C. The results are summarized in table I.

It is plain that treatment with the machine increased germination considerably in the case not only of legumes, but also of snapdragon, *Delphinium*, sweet marjoram, *Ipomoea*, okra, and lettuce.

TABLE I

GERMINATION OF SEED TREATED WITH MACHINE

KIND OF SEED	DURATION OF TEST IN DAYS	GERMINATION	
		Untreated	Treated
Alfalfa	10	51	99
Alfalfa	10	74	94
Bossiaea heterophylla	30	3	73
Bossiaea scolopendria	30	11	52
Clianthus Dampieri	10	0	80
White clover	10	70	94
Delphinium chinense	30	74	96
Dilliwynia ericifolia	30	2	77
Gleditschia	90	0	70
Ipomoea (average of 3 spp.)	10	34	71
Lathyrus (average of 3 vars.)	14	63	93
Lettuce (average of 11 vars.)	12	55	83
Sweet marjoram	30	54	96
Mustard (average of 2 vars.)	14	20	69
Okra	30	34	79
Perennial peas (average of 2 vars.)	10	62	87
Sweet peas (average of 5 vars.)	14	70	99
Platylobium trilobatum	30	13	64
Snapdragon	30	6	23
Vetch	10	77	92
Average		40.7	83.7
Increase due to treatment			43.7

It is not strictly correct, however, to call lettuce seeds hard-coated. That their germination is improved by treatment with the machine shows delay to be due to coat restrictions. The coat restrictions are removed also by soaking in water for 24 hours, hence it is likely that delay is caused by a reduction in rate of water absorption rather than by lack of oxygen. Whatever be the character of the seed coat, or coats, which interferes with germination, it disappears as the seed grows older. These points are well illustrated by table II. The data here presented support the statement made by various seedsmen, that two or three-year-old lettuce seed gives better germination than fresh. How this condition comes about is not known, but it probably depends on

changes in the permeability of the seed coat. It is certainly not a matter of embryo changes.

TABLE II

GERMINATION OF TREATED AND UNTREATED LETTUCE SEED

VARIETY	DURATION OF TEST IN DAYS	GERMINATION		
		Untreated	Treated with machine	Soaked in H_2O 24 hrs.
Black-seeded butter—				
1909	12	98	.....	.....
1910	12	99	.....	.....
1911	12	89	93	95
1912	12	67	97	100
Prize head—				
1909	12	98	.....	.....
1910	12	97	.....	.....
1911	12	84	96	97
1912	12	76	98	96

It appears, from a study of the records kept in this work, that not only the total germination, but also the rapidity or energy of germination is greater in treated than in untreated seeds. This is shown in table III.

TABLE III

RAPIDITY OF GERMINATION OF TREATED AND UNTREATED SEEDS*

Kind of seed	Germination after	Untreated	Treated
Alfalfa	3 days	48	98
White clover	3 "	69	89
Perennial peas	4 "	0	33
Perennial peas	4 "	24	80
Lupines	4 "	40	88
Delphinium	5 "	4	44
Sweet peas	4 "	0	70
Sweet peas	4 "	16	86

*For final germination percentage of these samples see table I.

Such rapidity of germination would clearly be of advantage in making the crop uniform in size and age, and in keeping down weeds. Indeed, it may be said truly that vigor of germination and vigor of the plants produced are more important than merely high germination percentage. Plants that get a good strong start

are more certain to be productive than those that for any reason are weak from the beginning.

Before this machine can become commercially practicable, experiments must be conducted to determine: (1) the possibility of substituting something else for needle points; (2) the proper distance these points should be from each other to give the best results for different sized seeds; (3) the pressure necessary to give the best germination for different kinds of seeds; for certain legumes a pressure of two to three pounds is necessary, for lettuce one pound or even less; (4) the effect of storage on the germination of treated seeds; (5) the germination of treated seeds in soil. To be effective in overcoming hard-coatedness, the needle point need only pass through the palisade layer and not entirely through the coat. Even with this slight deformation it is possible that bacteria and fungi can gain an entrance. That destruction by bacteria and fungi actually does take place was shown by JARZYMOWSKI (**17**) for seeds of *Ulex europaea*, lupines, and other large-seeded legumes which had been treated with the Wissinger machine. Red clover and *Lotus corniculatus* were the only ones whose germination in soil after treatment was not seriously reduced.

As to the germination in soil of seeds treated by the blowing method here described, there are not at present enough data on hand to justify the drawing of definite conclusions. Preliminary experiments seem to indicate for alfalfa seed, where the percentage of hard seed is high, that germination in soil is definitely better after treatment than before. Further investigation, of course, is necessary before this can be confirmed. In conclusion it may be stated that there was no serious crushing or cracking of seeds or seed coats by this machine.

The need of after-ripening

This is a condition which occurs, to mention only a few cases, in seeds of *Crataegus*, various conifers, *Fraxinus*, potato tubers, and lily-of-the-valley bulbs. For a discussion of the general situation and a résumé of the literature the reader is referred to the paper by ECKERSON (**10**) dealing with after-ripening in the seeds of *Crataegus*. The work to be discussed here had to do with the

germination of seeds of conifers, and specific reference will be made to a few of the more pertinent papers on the subject.

It is a matter of common knowledge that conifer seeds germinate slowly. It is also well known for several of them that as they grow older the rapidity of germination increases, up to at least the end of the first 6 months after they were gathered. SCHWAPPACH (**27**) states that in the fall seeds of *Abies* did not begin to show sprouts for 60 days, and required 40 days more before the test could be considered closed. In March they began almost immediately and finished in 20 days. The conclusion is natural that after-ripening takes place, and this, in fact, is assumed by workers who have recently attacked the problem. HILTNER and KINZEL (**14**), it is true, reasoning from results obtained by treating seeds of *Pinus Strobus*, *P. Peuce*, and *P. Cembra* with concentrated sulphuric acid, ascribed the delay to coat restrictions. LAKON (**20**) has made the objection that the tests on which these authors rely were too few and on too small a number of seeds. He repeated their experiments with the same three species of pine, but could obtain no forcing of germination. Untreated seeds took up water just as well as did the treated ones, even though their outer coats were hard. Careful determinations of the amount of water absorbed by untreated seeds of *Pinus sylvestris*, *P. Strobus*, *P. Peuce*, and *P. Cembra* showed that all of them reached nearly the maximum in 24–48 hours. Increases in weight after that time were practically negligible; hence it is clear that such seeds cannot be considered "hard-coated" like the seeds of legumes. Moreover, the cutting test, applied to these seeds, showed that all of them were damp, that is, had absorbed water. Increases in weight, therefore, were not due to a few easily swelling seeds. From these results LAKON concludes that conifer seeds are not, strictly speaking, "hard-coated," and that delay is due to conditions within the embryo.

Although LAKON found concentrated sulphuric acid ineffective, CORREVON states, in a paper published somewhat earlier, that weak acid (0.25 per cent acetic or 2 per cent phosphoric) increases the germination of seeds of *Juniperus Cedrus*.

SCHWAPPACH recommends cold storage for 14–30 days (he does not say how cold) for seeds of *Pinus Strobus*, followed by a germi-

nation temperature of 25° C. The common practice of layering various conifer seeds doubtless finds its justification in a shortening of the time required for germination. Low temperatures have also been used for preserving the vitality of conifer seeds. HAACK (**12**) dried seeds so they lost about 2 per cent in weight, then stored them in dry, air-tight containers on ice. CLEMENS (**9**) stored seeds in the refrigerator of a brewery in vessels containing sodium carbonate to absorb moisture and carbonic acid. Both investigators report that seeds thus stored remained viable longer than those kept under ordinary laboratory conditions.

In order to analyze the situation more carefully, the following series of experiments were conducted on seeds of various conifers.

1. Tests of untreated seeds.

2. Tests of seeds which had been in cold storage (3–5° C.): (*a*) in wet sand, (*b*) in weak solutions of hydrochloric acid, (*c*) in distilled water.

3. Tests were made with seeds which had been injected with weak hydrochloric acid or with water, by exhausting the air from them when they were in these liquids, and then restoring the pressure to normal. This was repeated at least three times for all seeds here spoken of as injected. Table IV summarizes the results obtained in series 1 and 2*a*.

TABLE IV

GERMINATION OF CONIFER SEEDS, UNTREATED AND AFTER STORAGE IN WET SAND AT 3–5° C.; PERCENTAGE GERMINATING AFTER ONE MONTH IN GERMINATOR

KIND OF SEED	DRY STORAGE UNTREATED				COLD WET STORAGE FOR			
					1 month	2 months	3 months	4 months
	Date of starting germination							
	Jan. 26	April 4	April 24	May 30	Feb. 26	Mar. 25	April 26	May 28
Cupressus macrocarpa	8	0	1	.....	15	27	40	36
Picea Menziesii	56	42	.....	23	70	60	.....	.....
Pinus austriaca	57	48	27	28	78	88	.....	64
Pinus Strobus	12	10	6	28	34	40	44	59

The results given in the table show that germination was definitely increased by cold wet storage for four kinds of conifers.

In no case does the maximum germination of seeds of a given kind from dry storage equal that of seeds of the same kind from cold wet storage. The effect of the cold wet storage is most noticeable in the cases of *Pinus Strobus* and *Cupressus macrocarpa*, where increases of 32 per cent and 31 per cent respectively were obtained. For all of the seeds here reported on, except those of *Pinus Strobus*, dry storage seemed to cause a decrease in viability. This can be seen from the first four columns of the table. Tests of *Pinus Strobus* were run for 60 days, the other three for 30 days.

TABLE V

GERMINATION OF SEEDS OF CONIFERS AFTER SOAKING IN WEAK ACID AND STORAGE AT 3–5° C.

KIND OF SEED AND LENGTH OF TIME IN COLD STORAGE	CONTROL	ACID (HCl)					WATER	
		Soaked			Injected			
		n/20,000	n/10,000	n/6,400	n/20,000	n/6,400	Soaked	Injected
Pinus Strobus—								
3 days	.....	44	58	53	66	54	.....	.....
6 "	.....	51	57	44	.....	.....	.....	.....
10 "	.....	53	65	67	.....	.....	63	.....
No cold storage	30	.....	.....	.....	.....	.....	50	68
Pinus austriaca—								
3 days	.....	64	.....	52	.....	.....	65	.....
6 "	.....	48	.....	42	.....	.....	59	.....
10 "	.....	35	.....	50	.....	.....	45	.....
No cold storage	52	54	.....	.....	.....	.....	.....	.....

The series of experiments shown in Table V was planned to determine whether delay in germination is due to an alkaline or neutral reaction of the embryo. It was thought that if such is the case, weak acid solutions would change the reaction sufficiently to cause growth to begin, when the seeds were placed in the proper conditions.

The results obtained for *Pinus Strobus* do not, however, bear out this theory. Seeds injected with distilled water gave 18 per cent better germination than those merely soaked in it, and slightly better than those injected with weak hydrochloric acid. It seems likely from this that delay is due merely to lack of water. When

this water was supplied, by long soaking or by forcible injection under pressure, germination was much improved.

Results for *Pinus austriaca* are less conclusive on this point. They do show, however, and the same is true for *P. Strobus*, that soaking in either water or weak acid gave greater germination than was obtained in the controls, 38 per cent and 13 per cent respectively. For *P. austriaca*, better results were obtained from short than from long soaking.

Referring again to table IV, it is possible that the increases in germination shown there were due not so much to the cold storage in itself as to the thorough infiltration of the seeds with water. There is need of much more work on this question before any definite conclusions can be drawn.

Exclusion of oxygen

No attempt will be made to review former work, since this has already been done by SHULL (**28**). Results presented in table VI seem to indicate that the germination of certain economic seeds is delayed for lack of oxygen. They also indicate the need of a detailed study of these seeds.

TABLE VI

GERMINATION OF SEEDS TREATED WITH OXYGEN OR HYDROGEN PEROXIDE

Kind of seed	Duration of test in days	Untreated	In 80 per cent oxygen	In 0.15 per cent H_2O_2
Dandelion	14	56	.....	72
Datura—				
Golden Queen	14	73	.....	70
Wrightii	14	20	.....	100
Lettuce—				
Grand Rapids	10	0	44	32
Martynia	20	0	90	80

Datura Wrightii was forced considerably by hydrogen peroxide, *Datura* Golden Queen not at all. Lettuce gave good results, but, as has been suggested, this is probably due to absorption of water.

Effect of frost on seeds

ATTERBERG (**2**) says that seeds of oats and barley harvested in Sweden after a heavy frost gave fair to good germination in the

laboratory, but in many cases no plants when sown in the field. Unpublished data obtained by EASTHAM in Canada show that the germination of oats grown in the prairie provinces is often seriously reduced by early frosts. He says, "as far as our observations go, a couple of degrees of frost in the milk stage are in many instances sufficient to ruin oats for seed. In the dough stage they are not nearly so susceptible, and when well ripened and dry stand considerable frost without serious injury." EASTHAM found also that such seed, germinating poorly when harvested, often improved with age. This seems to indicate in such cases the necessity for a period of after-ripening. Through the courtesy of the Canadian seed laboratory and two American seed houses the author has had the privilege of testing several samples of frosted oats. The results are summarized in table VII.

TABLE VII

GERMINATION OF OATS

VARIETY	UNTREATED; ON FILTER PAPER AT 20° C.	UNTREATED; SENDER'S TEST 18°–20° C.	HULLS OFF; ON FILTER PAPER AT 20° C.	HULLS REMOVED; ON COTTON IN PETRI DISHES AT 22° C.			
				80 per cent oxygen	60 per cent oxygen	40 per cent oxygen	20 per cent oxygen
Lincoln	85	40	79	.....	.....	.....	.....
Swedish	77	40	80	.....	.....	.....	.....
4920	34	35	38	31	25	38	33
5139	46	.....	.....	.....	.....	.....	.....
3477	56	43	.....	.....	.....	.....	.....
3974	45	42	.....	.....	.....	.....	.....
4948	89	62	94	93	97	95	96
5302	16	27	.....	.....	.....	.....	.....

The best germination was obtained from hulled seeds in oxygen, though the results are more clear-cut for no. 4948 than for no. 4920. No definite conclusions can be drawn as to what percentage of oxygen is most effective. It is noteworthy that two samples, nos. 3477 and 4948, show much better germination, 23 and 27 per cent respectively, than they did when tested in the Canadian Seed Laboratory six months earlier. This agrees well with the statement made above that frosted oats go through a process of after-ripening and improve in viability as they grow older. There is the same need of after-ripening in wild oats (*Avena fatua*), as has been

shown by ATWOOD. But referring to the table again, it will be seen that one sample, no. 5302, deteriorated in vigor as it grew older, since it gave a percentage of 27 when first tested and 11 per cent less 6 months afterward. Considering the results as a whole, it is clear that frosted oats are unreliable in performance and of very doubtful value for seeding purposes.

Another crop which sometimes suffers from frost is garden peas. Within the last two or three years the growing of garden peas for seed has become an important industry in Idaho and Montana. It has been found that certain of the late varieties grown there are injured by frost and the viability of the seed seriously impaired.

A study of 14 samples of such peas has shown that decrease in germination is probably due to two different causes, both of which, however, may be the effect of frost.

1. Actual injury to the embryo, especially the tip of the radicle. It has a whitish shriveled appearance and starts to grow very slowly, if at all.

TABLE VIII

GERMINATION OF PEAS

Variety	Coats on; 20°	Coats on; 25°	Coats off; 20°
Premium Gem	90	82	86
Nott's Excelsior 73186	68	.....	.....
" " 71531	98	100	.....
Telephone 885H	58	46	55
Gradus 913K	44	36	58
" 913S	70	.....	76
Dwarf Defiance 874C	56	.....	52
" " 874K	64	.....	96
" " 874H	78	.....	76
Alderman 912S	66	.....	.....
" 912K	82	.....	96
" 912T	56	.....	.....
" 912V	78	.....	92
Telegraph 68528	48	.....	50
Average	67	.....	74

2. The presence of fungi on or in the seed coat. That this actually decreases germination was shown by the work summarized in table VIII. With the coats on, the seeds of all the samples here reported on showed much fungus infection; with coats off,

very little or even none at all. Ten samples gave an average germination of 67 per cent with the coats on, and 74 per cent with the coats off, a difference of 7 per cent. Individual samples, such as Dwarf Defiance 874K, gave even more striking results.

The condition of garden peas, with reference to fungi, is approached more or less closely by that of a large number of other garden and flower seeds as is shown in table IX, summarizing the general results of this investigation, and in the discussion following.

Plants whose seeds show delayed germination, classified according to probable causes (the word "probable" is used advisedly, for while the evidence is convincing in some cases, it is much less so in others):

1. Hard-coatedness.—*Canna*, *Clianthus Dampieri*, *Delphinium*, *Erythrina*, *Hibiscus*, *Ipomoea* (4 spp.), *Lathryus*, *Lupinus*, sweet peas (4 vars.), snapdragon, alfalfa, sweet clover, white clover, lettuce (10 vars.), mustard (2 vars.), okra, sensitive plant, sweet marjoram, vetch, *Gleditschia*.

2. Frosted.—Oats, peas (8 vars.).

3. Need of after-ripening.—Wild cucumber, *Picea* (3 spp.), *Pinus* (2 spp.).

4. Exclusion of oxygen by the seed coat.—*Datura Wrightii*, *Martynia*.

5. Cause of delay not determined.—*Coix Lachryma*, feather grass, Pampas grass, asparagus, barley, blue grass, cardoon, celery, chives, dill, horehound, kaffir corn, leek, millet, parsley, parsnip, pepper, radish, rosemary, spinach, summer savory, thyme, *Aquilegia*, *Asparagus Sprengeri*, *Bignonia*, *Centaurea*, *Clematis*, dandelion, *Datura* Golden Queen, *Eschscholzia*, foxglove, heliotrope, *Helianthus*, hop, lavender, *Momordica*, *Nasturtium*, *Oenothera*, pansy, *Pentstemon*, *Primula*, *Salvia*, *Verbena*, *Abies Mertensiana*, *A. pectinata*, *Berberis*, *Betula alba*, *Cupressus horizontalis*, *C. macrocarpa*, *C. pyramidalis*, *Larix*. Further work would doubtless explain the cause of delay in many of these seeds and make the growing of plants from them a much simpler matter than it now is.

Very pertinent at this point are the results from the Minnesota Seed Laboratory for 1910 and 1911 (OSWALD **23**). Of field seeds 14 kinds were tested; of garden seeds 26 kinds. Of field seeds, for

TABLE IX

SHOWING THE NUMBER OF SPECIES AND VARIETIES TESTED, CLASSIFIED ACCORDING TO THE QUALITY OF GERMINATION AND THE PROBABLE CAUSES OF DELAY

SPECIES AND VARIETIES	GERMINATION				PROBABLE CAUSE OF DELAY						INFECTED WITH FUNGI
	Good	Poor or slow	None	Totals	Hard-coated-ness	Frost injury	Exclusion of oxygen by seed coat	Need of after-ripening	Not de-termined	Totals	
Ornamental grasses	.....	.....	3	3	.....	.....	.....	.....	3	3	.....
Field and garden plants	22	44	2	68	16	9	.....	.....	19	44	31
Ornamental flowering plants	11	35	3	49	19	.....	2	1	21	43	26
Shrubs and trees	.....	9	5	14	1	.....	.....	5	8	14	12
Totals	33	88	13	134	36	9	2	6	51	104	69

3 of the kinds, 50 per cent or more of the samples were below the government standard of germination. Of garden seeds, for 16 of the 26 kinds, 50 per cent or more of the samples were below standard. Mention should be made also of work by BROWN (**5**) on the germination of packeted vegetable seeds. He found that the average germination of box vegetable seeds put up by 60 firms for four years was 60.5 per cent. The lowest average for any firm was 36.5 per cent, the highest 81.5 per cent. The average germination of packeted vegetable seeds put up by 20 mail-order houses in 1911 was 77.5 per cent (lowest average 76.2 per cent, highest 77.5 per cent). Just what these figures signify is not clear. There are three possibilities: (1) the seeds were poor because of the seedsman's dishonesty or carelessness; (2) the seeds were poor because it is not possible with present methods to produce better ones; if so, the government standard is, now at least, too high and methods of production need improvement; (3) the seeds seemed poor because present methods of making germination tests do not always adequately determine the value of a given sample. In the writer's opinion, the responsibility for low test must be shared about equally by all three, though the first is a less important factor than it was a few years ago.

6. Plants whose seeds were found infected with fungi.—Feather grass, asparagus, beggar weed, buckwheat, cardoon, celery, chives, sweet clover, dill, kaffir corn, leek, millet, oats, parsley, peas (12 vars.), pepper, radish, rosemary, spinach, thyme, vetch, *Aquilegia*, *Asparagus Sprengeri*, *Bignonia*, *Clematis*, *Clianthus Dampieri*, wild cucumber, dandelion, *Datura* Golden Queen, *D. Wrightii*, *Helianthus*, hop, *Ipomoea* (4 spp.), *Lathyrus*, lavender, *Nasturtium*, pansy, sweet peas (4 vars.), *Primula*, *Verbena*, *Abies Mertensiana*, *A. pectinata*, *Berberis*, *Cupressus horizontalis*, *C. macrocarpa*, *C. pyramidalis*, *Picea excelsa*, *P. Menziesii*, *P. rubra*, *Pinus austriaca*, *P. Strobus*.

The species and varieties tested were 134, but 30 of these are omitted from the second section of table IX; 29 of these germinated rapidly and well, and one other, on account of bad infection with fungi, showed not delayed but definitely poor germination; 69, or 51.4 per cent of the total, were found more or less infected with

fungi. This point was determined for each kind of seed not more than two days after the test began. All filter paper was boiled 5–10 minutes before being used and kept moist with distilled water during the test. Repeated washing of seeds and removal to fresh filter paper showed that in all cases infection came from the seeds, not from the paper.

There is no intention here of implying that seedsmen in general purposely put on the market seeds low in vitality or badly infected with fungi. It does seem clear, however, that there is need of closer supervision by the seedsmen themselves of all stages of the process of seed production; alternation of crops to avoid soil-infection, cultivation, harvesting, threshing, cleaning, storage; all of these need close attention if seed of the best quality is to be produced. The most candid way in which to approach the whole question is to admit that seed analysts, seed-growers, and seed merchants do not at present know a number of things they need to know in reference to the question of fungus infection of seeds, and to all the other questions considered in this paper. The whole matter constitutes an extremely complex physiological and pathological problem, with very practical aspects, the solution of which can be brought about only by careful study from several different points of view. To be specific, the following lines for investigation may be suggested:

1. The relation of germinator tests to the actual vegetation of seeds in the soil. This should be studied through a period of several years.

2. The relation of fungi on or inside of seeds to the germination of such seeds in soil. At the risk of seeming to repeat unnecessarily, the writer wishes to say that in his opinion the importance of this problem is only poorly appreciated in this country. Some recognition of the dangers accompanying fungus infection of seed has appeared of late in the work of BOLLEY and others in the United States, and in German agricultural literature. APPEL (1), writing on the relation of pathology to seed control, says that in seed-testing stations, pains should be taken to give judgment as to the presence of spores of plant diseases on seeds to be examined. It is his opinion, further, that in comparative field tests more

attention must be given than formerly to pathological phenomena. Observations on this point should be given along with other data from the experiment.

3. The causes of delayed germination in asters, certain hardy perennials, labiates, ornamental grasses, cucurbits, conifers, frosted oats, *Betula*, and *Berberis*.

4. The value of hard seeds of legumes when planted in the soil.

5. The relation of any or all of the causes of delayed germination to the vigor of the plants produced. It is not enough that a given lot of seeds shall be free from impurities; it is not even enough that it shall give a high germination percentage. It must, above all, give rise to vigorous productive plants, when planted in field conditions. Consequently, any knowledge which will teach us how to grow such seeds and how to know poor seeds is of the greatest practical importance.

Summary

1. Hard-coated seeds of legumes, and seeds of *Delphinium*, *Ipomoea*, lettuce, mustard, okra, sweet marjoram, and snapdragon can be forced to more rapid germination by being blown against needle points.

2. For two varieties of lettuce it is shown that the seed improves in viability as it grows older, up to the end of at least the fourth year. This improvement is probably due to increased permeability of the inner seed coat to water.

3. Cold storage in wet sand increased the germination seeds of *Pinus Strobus* by 32 per cent, of *Cupressus macrocarpa* by 31 per cent. Delayed germination of conifer seeds, more especially those of *Pinus Strobus* and *P. austriaca*, seems to be due to lack of water intake, and not to an alkaline or neutral reaction of the embryo. This statement is supported by the fact that seeds injected with distilled water gave better germination than those merely soaked in water or in weak acid at the temperature of melting ice. Any kind of soaking or injection gave 13–38 per cent better germination than was obtained with the controls.

4. Certain samples of frosted oats improve in germinating power as they grow older, others deteriorate.

5. Certain late varieties of western-grown garden peas germinate poorly. This is shown to be due to one or both of two causes: (*a*) actual frost injury to the embryo; (*b*) the presence of fungi on or in the seed coat or inside of it.

6. Seeds of 51.4 per cent of all species and varieties examined showed fungi on the seed coat within two days after being put to germinate.

The writer is indebted to Dr. WILLIAM CROCKER for many suggestions and criticisms.

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SPECIFIC ACTION OF ORGANIC COMPOUNDS IN MODIFYING PLANT CHARACTERISTICS; METHYL GLYCOCOLL VERSUS GLYCOCOLL

OSWALD SCHREINER AND J. J. SKINNER

(WITH FOUR FIGURES)

The effect on plant growth of a large number of soil organic compounds and other organic substances has been tested in this laboratory from time to time. The action of the two compounds glycocoll and methyl glycocoll on plants is very interesting, the former being beneficial, while the latter is harmful to growth and affects the plants in a peculiar way.

The utilization of certain nitrogenous compounds by plants, some having a beneficial effect and replacing nitrates in their action, and others having harmful effects, producing peculiar characteristics, leaves but little doubt that organic compounds in soils or nutrient solutions are absorbed directly by the roots of plants and enter into the cells, reacting with the cell contents and producing effects which differ according to the nature of the compound absorbed. The process is connected with and is a part of the general metabolic processes of plants. The absorbed material passes through the membranes possessing these properties of absorption, and reacts on the cell contents in a favorable or unfavorable manner, influencing the life processes of the plant itself. Glycocoll, a nitrogenous compound having a definite chemical structure, is shown to have been absorbed or used by the plant to build up its tissue, while the related compound, methyl glycocoll, also nitrogenous but having a different chemical structure, is absorbed by the plants and has an unfavorable influence, causing decreased growth, and a peculiar twisted lateral growth of the leaf of the plant.

The properties of plants of absorbing the mineral constituents from the nutrient or soil solution do not differ in respect to the absorption of the organic constituents from the solution. In the case of the methyl glycocoll the greatest harmful effect was noted

in those solutions where there was the greatest absorption of inorganic nutrients, and while no methods are available to study the absorption of this organic compound, it seems justifiable to assume that the cultures also absorbed a greater amount of organic constituent from the solution, while in other solutions where the absorption of nutrients was small the harmfulness of the methyl glycocoll was also only slight, indicating that a small amount was absorbed.

It has been shown in this laboratory and elsewhere that plant roots can affect organic substances externally, and it is therefore possible that organic substances may also influence the plant itself through this external action; however, in the majority of cases that have come under our observation the compounds have been absorbed, for in such cases as could be tested they disappeared from the solution and had their effects on the plant tops either favorably or unfavorably, as the case may be.

In the case of dihydroxystearic acid the normal metabolism is greatly disturbed.[1] This is shown by the difference in the absorption of the separate nutrients, phosphate, nitrate, and potash, a proportionately greater nitrate consumption being evident with the plants affected by dihydroxystearic acid. Such a change in metabolism could be explained only on the assumption that the compound produced a reaction within the plant after absorption from the solution.

The specific effects produced by organic compounds must also be taken into consideration. For instance, cumarin produces greatly stunted tops, with short, broad leaves and much distorted and thickened stems in the case of wheat plants. Quinone, on the other hand, produces long, slender, thin plants. The different reactions of the plants to these two compounds must be due to the direct absorption of the compound accompanied by a disturbed metabolism. Moreover, the cumarin-affected plants absorb relatively more phosphate, the quinone plants relatively more potash.[2]

Furthermore, plants grown in guanidine solutions develop small spots of a bleached appearance which grow and spread, producing

[1] SCHREINER, O., and SKINNER, J. J., Some effects of a harmful organic soil constituent. BOT. GAZ. **50**:161. 1910.

[2] ———, The toxic action of organic compounds as modified by fertilizer salts. BOT. GAZ. **54**:31. 1912.

a weakened plant, the leaves of which break at the stem, wilt, and die, the roots remaining unaffected.[3] This is explainable only on the assumption of its absorption by the plant and its action on the plant protoplasm.

The cases cited of the beneficial and harmful action of organic compounds, many having specific characteristics, are sufficient to show that organic substances, like inorganic poisons, are absorbed and react with the protoplasm of the plant.

The effect of methyl glycocoll on plants has not previously been determined. In the early experiments[4] of this laboratory glycocoll was shown to be beneficial to wheat seedlings in distilled water cultures. Concentrations of 1 to 1000 parts per million were used. The tops were increased in all the cultures; the roots were slightly injured by the higher amounts.

HANSTEEN[5] showed that glycocoll was slightly harmful to *Lemna*, and that from it were produced proteins. In MOLLIARD'S[6] experiments beneficial results were secured in water cultures growing radishes. The experiments of HUTCHINSON and MILLER[7] with water cultures were doubtful. One of their pea cultures showed that the nitrogen content of the plant was increased by the use of glycocoll, although a slight decrease was noticed in another culture.

The experiments of BOROWIKOW[8] with *Helianthus* indicate that glycocoll retards growth. DACHNOWSKI and GORMLEY,[9]

[3] SCHREINER, O., and SKINNER, J. J., The effect of guanidine on plants. Bull. Torr. Bot. Club **39**:535. 1912.

[4] SCHREINER, O., REED, H. S., and SKINNER, J. J., Certain organic constituents of soils in relation to soil fertility. Bull. 47. Bur. Soils, U.S. Dept. Agric. 1907.

[5] HANSTEEN, B., Om Aeggehvidesynthese i den gronne phanerogame Plante. Vidensk. Skrift. no. 3. 1898; Über Eiweissynthese in grünen Phanerogamen. Jahrb. Wiss. Bot. **33**:417. 1899.

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[7] HUTCHINSON, H. B., and MILLER, N. H. J., The direct assimilation of inorganic and organic forms of nitrogen by higher plants. Centralbl. Bakt. **30**:513. 1911.

[8] BOROWIKOW, G. A., Über die Ursachen des Wachstums der Pflanzen. Biochem. Zeitschr. **50**:119. 1913.

[9] DACHNOWSKI, A., and GORMLEY, R., The physiological water requirements and growth of plants in glycocoll solutions. Amer. Jour. Bot. **1**:174. 1914.

working with a number of plants in water cultures, have shown that glycocoll is generally beneficial. It is pointed out that the process of absorption of glycocoll is not connected with the transpirational water loss, but with the differential permeability of the absorbing root cells, with the efficiency of the nutrient metabolism characteristic of the plant, and the amount of water retained within the plants.

Glycocoll ($CH_2 \cdot NH_2 \cdot COOH$) is amidoacetic acid and is one of the simpler degradation products found where decomposition is occurring. This primary degradation product of protein appears in the decomposition of plant remains and exists in considerable quantities in the tissue and seed of many plants. It is obtained synthetically by chemical processes in a number of ways.

Methyl glycocoll ($CH_2 \cdot NH \cdot CH_3 \cdot COOH$) differs in its chemical structure from glycocoll, not only in that it contains the methyl group CH_3, but also in that the amido group NH_2 is thereby changed to an imido group NH. Methyl glycocoll, as well as glycocoll, is a nitrogen-containing body; it is made synthetically and is not a simple protein body.

Effect of glycocoll

In this investigation the effect of glycocoll on growth in culture solutions was studied by growing wheat seedlings in solutions of calcium acid phosphate, sodium nitrate, and potassium sulphate. A large number of cultures was used; some consisted of the salts used singly, some of combinations of two salts, and others of all three salts used in different proportions. The total number of combinations used can be obtained from table IV, and from the discussion of the other tables. The composition of the solutions is given in the respective tables. Each solution was contained in a wide-mouth bottle holding 250 cc., and the solution was changed every three days, the old being replaced by fresh solution. The solutions were prepared by dissolving definite amounts of salts in carbon-treated distilled water. Two bottles of each solution were prepared, one to serve as a control, while to the other was added 50 ppm. of glycocoll. Each culture jar grew 10 wheat seedlings, supported in notched corks. The seedlings were placed in

the solutions when about 2 cm. tall, and grew for 12 days. The removal of salts was studied by analyzing the solutions for phosphate, nitrate, and potash, and the growth was observed and compared with its control, and the weight of green plants taken at the end of the experiment. The plants grew in these solutions from February 7 to February 19, 1914.

As the plants grew in the solutions, it was apparent that the glycocoll affected growth differently in the solutions containing different nutrient salts. The growth was increased much more by glycocoll in the solutions which contained no sodium nitrate than in those which contained large amounts of nitrate, as compared with the respective controls. This will be apparent from the weights of the green plants given in the accompanying tables.

TABLE I

EFFECT OF GLYCOCOLL IN CULTURE SOLUTIONS CONTAINING VARYING AMOUNTS OF PHOSPHATES AND POTASH AND NO NITRATE

COMPOSITION OF CULTURE SOLUTIONS			GREEN WEIGHT	
P_2O_5	NH_3	K_2O	Without glycocoll	With 50 ppm. glycocoll
ppm.	ppm.	ppm.	gm.	gm.
80	0	0	1.01	1.05
72	0	8	1.26	1.55
64	0	16	1.53	1.85
56	0	24	1.50	1.95
48	0	32	1.55	2.05
40	0	40	1.47	2.00
32	0	48	1.39	2.05
24	0	56	1.50	1.95
16	0	64	1.65	1.70
8	0	72	1.40	1.90
0	0	80	1.37	1.65

Table I gives the green weight of the plants in solutions without and with glycocoll. The first three columns give the composition of each solution in parts per million P_2O_5 as calcium acid phosphate, NH_3 as sodium nitrate, and K_2O as potassium sulphate. The fourth column gives the green weight of the plants grown in solutions without glycocoll, and the last column the weight of the plants grown in solutions with 50 ppm. glycocoll.

An examination of the table shows that glycocoll, in those solutions which contained no nitrate, but varying amounts of

phosphate and potash, produced an increased growth in every culture. The total weight of the 11 normal cultures was 15.63 grams, and the weight of the glycocoll cultures was 19.70 grams, an increase of 26 per cent.

In table II are given the cultures which were composed of 8 ppm. of NH_3 as nitrate, and varying amounts of phosphate and potash.

TABLE II

EFFECT OF GLYCOCOLL IN NUTRIENT SOLUTIONS CONTAINING VARYING AMOUNTS OF PHOSPHATE AND POTASH AND 8 PPM. OF NH_3 AS NITRATE

Composition of culture solutions			Green weight	
P_2O_5	NH_3	K_2O	Without glycocoll	With 50 ppm. glycocoll
ppm.	ppm.	ppm.	gm.	gm.
72	8	0	1.48	1.65
64	8	8	1.73	2.05
56	8	16	2.20	2.09
48	8	24	2.40	2.43
40	8	32	2.37	2.70
32	8	40	2.34	2.35
24	8	48	1.30	1.45
16	8	56	2.20	2.25
8	8	64	2.35	2.40
0	8	72	1.90	2.05

The growth in this series of solutions was slightly better where glycocoll was added. An examination of the green weight columns of the table will show that nearly all the glycocoll cultures were slightly heavier. The total weight of the 10 cultures without glycocoll was 20.27 grams, against 21.42 grams for the cultures with glycocoll. This is an increase of 6 per cent.

The effect of glycocoll on growth in nutrient solutions containing still larger amounts of sodium nitrate is given in table III. These solutions, like the ones in the previous tables, are composed of varying amounts of phosphate and potash, but each contains 16 ppm. of NH_3 as nitrate.

In some of these solutions the glycocoll has slightly increased the growth, and in others the growth is slightly below the normal culture. The total weight of the 9 cultures without glycocoll was

20.75 grams, against 21.60 grams for the glycocoll, an average increase of only 4 per cent.

TABLE III

EFFECT OF GLYCOCOLL IN CULTURE SOLUTIONS CONTAINING VARYING AMOUNTS OF PHOSPHATE AND POTASH AND 16 PPM. NH_3 AS NITRATE

Composition of culture solutions			Green weight	
P_2O_5	NH_3	K_2O	Without glycocoll	With 50 ppm. glycocoll
ppm.	ppm.	ppm.	gm.	gm.
64	16	0	1.33	1.45
56	16	8	1.90	2.05
48	16	16	2.40	2.25
40	16	24	2.50	2.60
32	16	32	2.62	2.50
24	16	40	2.50	2.65
16	16	48	2.90	2.85
8	16	56	2.30	3.00
0	16	64	2.30	2.25

The beneficial effect of glycocoll in the solutions given in tables I, II, and III was most marked in those solutions containing no nitrate (table I). The effect was very slight in solutions containing 8 and 16 ppm. of NH_3 as nitrate, which indicates that the function of glycocoll in the nutrient solution is the same as that of nitrate; that is, that it seems to be absorbed by the plants and can take the place of nitrate in its effect on growth.

A large number of other solutions containing larger amounts of nitrate, up to 80 ppm., were employed in this experiment. No beneficial effect from glycocoll was observed in any of the cultures; on the other hand, some slight reductions in growth in some of these solutions was noted, but there was no marked harmful effect.

Absorption of nutrient salts as affected by glycocoll

The absorption of nutrients from the various solutions was determined, as mentioned before, by analyzing the cultures for nitrates immediately at the end of each 3-day period, and the phosphate and potassium on a composite of the solutions from the four changes. The colorimetric methods for determining small

amounts of salts, as described in Bulletin 70 of the Bureau of Soils, were employed in this investigation.

Considering first the absorption of phosphate, the entire set of cultures containing no glycocoll absorbed 177.5 mg. of P_2O_5, while the similar set of cultures with glycocoll absorbed 233 mg. The relative absorption of potash was somewhat similar. In the solutions without glycocoll, 613.7 mg. of K_2O was removed, while from the cultures with glycocoll 623.5 mg. was absorbed. With both phosphate and potash, the glycocoll cultures removed more than the normal cultures, which was to be expected, as the glycocoll cultures made a larger growth. However, the absorption of nitrate was less by the glycocoll than the normal cultures. The set of cultures containing no glycocoll removed 544.7 mg. NH_3, and the cultures with glycocoll only 320.5 mg. NH_3. The removal of less nitrate from solutions containing glycocoll is also contributing evidence that the plants use the glycocoll in building tissue, as it would use the nitrate in this particular function.

The effect of glycocoll seems to be the same as that of creatinine,[10] creatine, histidine, arginine,[11] asparagine,[12] xanthine, hypoxanthine, and nucleic acid,[13] all nitrogenous compounds and shown to be beneficial to growth, especially in the absence of any other form of nitrogen. These compounds replace the effect of nitrates on plants and are used as such by the plant.

In recent years it has been demonstrated that plants not only use nitrogen in the form of nitrates and ammonia, but that they can also use nitrogen in the form of complex organic compounds.[14] The

[10] SKINNER, J. J., Beneficial effect of creatinine and creatine on growth. BOT. GAZ. **54**:152. 1912.

[11] ———, Effect of histidine and arginine as soil constituents. Eighth Internat. Cong. Applied Chem. **15**:253. 1912.

[12] ———, and BEATTIE, J. H., Effect of asparagine on absorption and growth. Bull. Torr. Bot. Club **39**:429. 1912.

[13] SCHREINER, O., and SKINNER, J. J., Experimental study of the effect of some nitrogenous soil constituents on growth. Nucleic acid and its decomposition products. Plant World **16**:45. 1913.

[14] SCHREINER, O., Symposium on soils at the 1911 meeting of the A.A.A.S. Science **36**:577. 1912.

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action of these and a number of other nitrogenous compounds has been tested in this laboratory, and it has been found that these compounds are used as a source of nitrogen for the plant, without any transformation into ammonia, nitrites, or nitrates, and that the plant absorbs and uses them in preference to nitrate.

Effect of methyl glycocoll

The effect of methyl glycocoll on growth was studied in a similar way as was glycocoll; that is, wheat plants were grown in nutrient culture solutions composed of fertilizer salts, used singly and in combination of two and three salts. The methyl glycocoll was used in the cultures in amounts of 50 ppm., the same concentration as in the glycocoll experiments. The composition of the 66 nutrient solutions used are given in table IV, together with the green weight of the plants, grown in the solutions without and with methyl glycocoll. The plants grew from January 14 to January 26; within this time the solutions were changed and replaced by fresh solutions of the same composition four times in 3-day periods.

By an examination of table IV it will be seen that the methyl glycocoll, unlike the glycocoll, caused a decrease in growth. This is true in the entire set of cultures except four. The total green weight of the 66 cultures containing nutrient salts, but no methyl glycocoll, was 128.03 grams; while the total green weight of the similar set of cultures containing methyl glycocoll was 99.3 grams, a reduction in growth of 33 per cent.

The root growth of the plants in the methyl glycocoll solutions was shorter and did not have as healthy an appearance as the roots of the plants in the solutions which contained only the nutrient salts.

The tops, aside from being lighter in weight, were abnormal in appearance. The tops did not stand upright, but were twisted and grew in a lateral direction. This was true of each culture in the entire set, regardless of its content of nutrient salts. These physiologically disturbed plants had a pale green color, in contrast to the dark green of the normal cultures. The effect of this substance resembles somewhat that of cumarin,[15] which causes

[15] SCHREINER, O., and SKINNER, J. J., The toxic action of organic compounds as modified by fertilizer salts. BOT. GAZ. **54**:31. 1912.

TABLE IV

EFFECT OF METHYL GLYCOCOLL ON GROWTH OF WHEAT SEEDLINGS IN CULTURE SOLUTIONS OF CALCIUM ACID PHOSPHATE, SODIUM NITRATE, AND POTASSIUM SULPHATE

No.	COMPOSITION OF CULTURE SOLUTIONS			GREEN WEIGHT	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With 50 ppm. methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
1	80	0	0	1.02	0.95
2	72	0	8	1.27	1.30
3	72	8	0	1.32	1.20
4	64	0	16	1.32	1.25
5	64	8	8	1.47	1.45
6	64	16	0	1.20	1.20
7	56	0	24	1.35	1.30
8	56	8	16	1.80	1.55
9	56	16	8	1.85	1.45
10	56	24	0	1.35	1.20
11	48	0	32	1.15	1.30
12	48	8	24	1.95	1.40
13	48	16	16	2.25	1.70
14	48	24	8	1.75	1.45
15	48	32	0	1.60	1.10
16	40	0	40	1.47	1.25
17	40	8	32	2.07	1.55
18	40	16	24	2.19	1.75
19	40	24	16	2.14	1.40
20	40	32	8	1.95	1.50
21	40	40	0	1.60	1.15
22	32	0	48	1.52	1.35
23	32	8	40	2.12	1.50
24	32	16	32	2.42	1.75
25	32	24	24	2.44	1.70
26	32	32	16	2.02	1.72
27	32	40	8	1.90	1.60
28	32	48	0	1.40	1.30
29	24	0	56	1.42	1.32
30	24	8	48	2.09	1.82
31	24	16	40	2.44	1.84
32	24	24	32	2.22	1.80
33	24	32	24	2.42	1.80
34	24	40	16	2.32	1.70
35	24	48	8	1.89	1.40
36	24	56	0	1.49	1.42
37	16	0	64	1.47	1.32
38	16	8	56	2.30	1.42
39	16	16	48	2.55	1.70
40	16	24	40	2.54	1.59
41	16	32	32	2.54	1.70
42	16	40	24	2.24	1.60
43	16	48	16	2.37	1.55
44	16	56	8	1.97	1.57
45	16	64	0	1.70	1.40
46	8	0	72	1.57	1.37
47	8	8	64	2.07	1.59

TABLE IV—*Continued*

No.	Composition of culture solutions			Green weight	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With 50 ppm. methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
48	8	16	56	2.34	1.89
49	8	24	48	2.54	1.70
50	8	32	40	2.90	1.70
51	8	40	32	2.42	1.65
52	8	48	24	2.45	1.60
53	8	56	16	2.22	1.70
54	8	64	8	2.32	1.70
55	8	72	0	1.52	1.45
56	0	0	80	1.32	1.43
57	0	8	72	1.95	1.45
58	0	16	64	2.29	1.50
59	0	24	56	2.27	1.55
60	0	32	48	2.30	1.65
61	0	40	40	2.25	1.65
62	0	48	32	2.20	1.67
63	0	56	24	2.32	1.67
64	0	64	16	1.96	1.50
65	0	72	8	1.76	1.40
66	0	80	0	1.35	1.28

distorted stems and produces broad twisted leaves. Cumarin, however, does not cause a pale green leaf when grown in similar nutrient solution as does the methyl glycocoll. A set of normal and methyl glycocoll cultures is shown in figs. 1 and 2. The characteristic action is distinctly shown in the plants in fig. 2; the leaves are twisted and broad, which is in striking contrast to the straight upright plants of the normal cultures in fig. 1.

In analyzing the green weight figures given in table IV, it is interesting to note that the methyl glycocoll has reduced growth more in some of the cultures than in others, which will be brought out in the tables that follow. A close examination shows that the reduction was most in those solutions which normally produce the largest growth; that is, the difference between the green weight of specific cultures of some compositions without and with methyl glycocoll is greater where the growth is greater in the solutions containing only the nutrient salts.

The 66 cultures given in table IV permit of better discussion when they are arranged according to the triangular scheme upon which their composition depends. This scheme has been fully

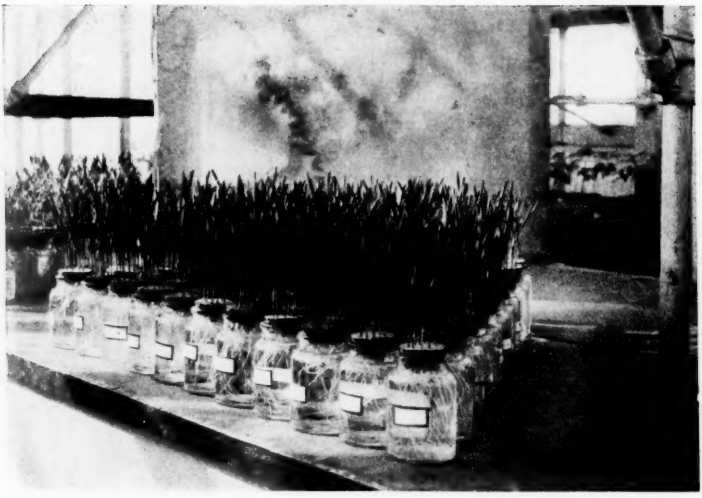

FIG. 1.—Wheat plants growing in nutrient solutions

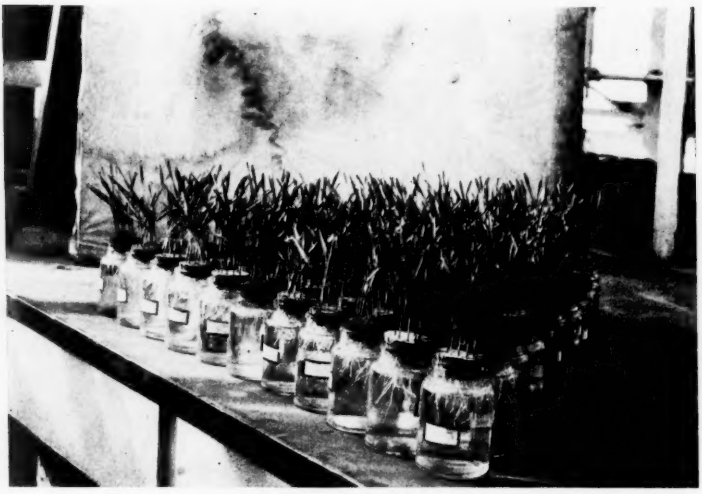

FIG. 2.—Wheat plants growing in nutrient solutions with 50 ppm. of methyl glycocoll.

presented in an earlier paper[16] and needs no further exposition here. As shown in this earlier paper, the maximum growth takes place in the middle region of the lower part of the diagram; that is, in those cultures which contain phosphate between the limits 8–24 ppm., and nitrate and potash between the limits 24–48 ppm. These are the cultures represented by group *a* in fig. 3, which is here

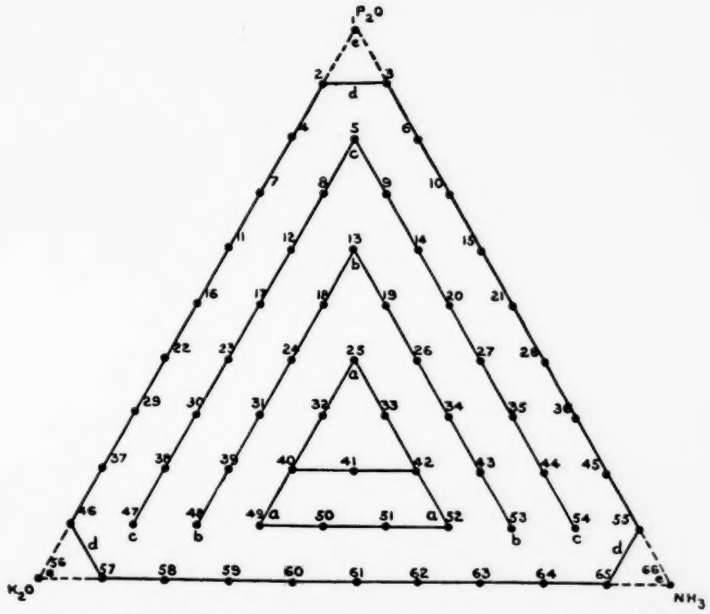

FIG. 3.—The arrangement of the culture solutions in groups: *a*, *b*, *c*, three fertilizer element groups; *d*, two fertilizer element group; *e*, one fertilizer element group.

again reproduced to make the discussion clear. It has been shown that this group *a* has the largest growth, and the greatest absorption of nutrients by the growing plants also takes place in this group. Both growth and absorption become less successively in groups *b*, *c*, and *d*. The latter group contains only two of the fertilizer elements in any one solution, and the growth in these is

[16] SCHREINER, O., and SKINNER, J. J., Ratio of phosphate, nitrate, and potassium on absorption and growth. BOT. GAZ. 50: 1. 1910.

considerably poorer than in those containing three of the fertilizer elements.

If we consider now the results of table IV in the light of these facts and in the same groupings, some interesting facts regarding the effect of the methyl glycocoll are revealed.

In table V are given the cultures of group *a*, which as a group normally produces the largest growth and the greatest absorption. The number of the culture is given in the first column; the third, fourth, and fifth columns give the composition of the solution; the sixth the green weight of plants grown in solution without methyl glycocoll; and the last column the green weight of plants with methyl glycocoll. There is considerable difference in growth in these two sets of cultures, as is seen in the table.

TABLE V

GROWTH OF WHEAT PLANTS IN THE BEST CULTURE SOLUTIONS OF GROUP *a* WITHOUT AND WITH METHYL GLYCOCOLL

No.	Composition of culture solutions			Green weight	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
25	32	24	24	2.44	1.70
33	24	24	32	2.22	1.80
34	24	32	24	2.42	1.80
40	16	24	40	2.54	1.59
41	16	32	32	2.54	1.70
42	16	40	24	2.24	1.60
49	8	24	48	2.54	1.70
50	8	32	40	2.90	1.70
51	8	40	32	2.42	1.65
52	8	48	24	2.45	1.60

The total green weight of the 10 cultures without methyl glycocoll was 24.71 grams, against 16.84 grams for the methyl glycocoll cultures, a reduction of 32 per cent. It is also interesting to compare the absorption of phosphate, nitrate, and potassium in these two sets of cultures. The 10 normal cultures absorbed 28.9 mg. of P_2O_5, NH_3, and K_2O, and the 10 cultures with the methyl glycocoll absorbed 20.8 mg. That is, the methyl glycocoll cultures absorbed 28 per cent less nutrients than the normal cultures.

We will next consider the solutions of group *b*, which contained more unequal ratios and which have previously proved to have a poorer physiological influence on plants as manifested by the growth. The composition of these solutions together with the green weights of the two sets of cultures is given in table VI.

TABLE VI

GROWTH OF WHEAT PLANTS IN CULTURE SOLUTIONS OF GROUP *b* WITHOUT AND WITH METHYL GLYCOCOLL

No.	Composition of culture solutions			Green weight	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
13	48	16	16	2.25	1.70
18	40	16	24	2.19	1.75
19	40	24	16	2.14	1.40
24	32	16	32	2.42	1.75
26	32	32	16	2.02	1.72
31	24	16	40	2.44	1.84
34	24	40	16	2.32	1.70
39	16	16	48	2.55	1.70
43	16	48	16	2.37	1.55
48	8	16	56	2.34	1.89
53	8	56	16	2.22	1.70

The figures show that methyl glycocoll produced less growth than the normal solutions. The green weight of the 11 normal cultures was 26.10 grams, against 18.70 grams total green weight for the methyl glycocoll cultures, a reduction of 29 per cent due to the methyl glycocoll. The 11 normal cultures absorbed 27.43 mg. of P_2O_5, NH_3, and K_2O, while the methyl glycocoll cultures absorbed 19.45 mg., a reduction of 22 per cent in nutrients absorbed. It is interesting to note that methyl glycocoll reduced both the growth and absorption less in these cultures of group *b* than in those of group *a* given in table V.

In table VII is given the effect of methyl glycocoll in the solutions of group *c*, which have been shown to be not as suitable for plant development as those given in the two preceding tables.

The total green weight of the 15 cultures without methyl glycocoll was 29.50 grams, and the total green weight of the same solutions containing methyl glycocoll was 22.95 grams, a reduction

of 22 per cent in growth. The absorption of P_2O_5, NH_3, and K_2O from these solutions for the 15 normal cultures was 18.2 mg., against 15.5 mg. for the total number of cultures containing methyl glycocoll, a reduction of 15 per cent. Again it is seen that in these cultures where absorption and growth is small the methyl glycocoll is less harmful.

TABLE VII

GROWTH OF WHEAT PLANTS IN CULTURE SOLUTIONS OF GROUP *c* WITHOUT AND WITH METHYL GLYCOCOLL

No.	COMPOSITION OF CULTURE SOLUTIONS			GREEN WEIGHT	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
5	64	8	8	1.47	1.45
8	56	8	16	1.80	1.55
9	56	16	8	1.85	1.45
12	48	8	24	1.95	1.40
14	48	24	8	1.75	1.45
17	40	8	32	2.07	1.55
20	40	32	8	1.95	1.50
23	32	8	40	2.12	1.50
27	32	40	8	1.90	1.60
30	24	8	48	2.09	1.82
35	24	48	8	1.89	1.40
38	16	8	56	2.30	1.42
44	16	56	8	1.97	1.57
47	8	8	64	2.07	1.59
54	8	64	8	2.32	1.70

In table VIII are given the green weights of plants grown in solution containing only two nutrient salts (group *d*), with and without methyl glycocoll. Solutions containing only two salts produce less growth than solutions containing three salts, in any proportion.

The total growth of the 27 cultures without methyl glycocoll was 44.72 grams green weight, against 37.32 grams for the cultures with methyl glycocoll, a reduction of only 16 per cent.

From tables V, VI, VII, and VIII it is apparent that growth was greatest in the cultures of group *a*, and that the greatest amount of absorption took place in these solutions. The harmful effect of methyl glycocoll was also most marked in this group of solutions. A comparison of tables VI, VII, and VIII shows that those solutions

in which less absorption took place and less growth was produced, the effect of the organic compound was also less.

TABLE VIII

GROWTH OF WHEAT PLANTS IN CULTURE SOLUTIONS OF GROUP *d* COMPOSED OF TWO FERTILIZER ELEMENTS, WITHOUT AND WITH METHYL GLYCOCOLL

No.	Composition of culture solutions			Green weight	
	P_2O_5	NH_3	K_2O	Without methyl glycocoll	With methyl glycocoll
	ppm.	ppm.	ppm.	gm.	gm.
2	72	0	8	1.27	1.30
4	64	0	16	1.32	1.25
7	56	0	24	1.35	1.30
11	48	0	32	1.15	1.30
16	40	0	40	1.47	1.25
22	32	0	48	1.52	1.35
29	24	0	56	1.42	1.32
37	16	0	64	1.47	1.32
46	8	0	72	1.57	1.37
57	0	8	72	1.95	1.45
58	0	16	64	2.29	1.50
59	0	24	56	2.27	1.55
60	0	32	48	2.30	1.65
61	0	40	40	2.25	1.65
62	0	48	32	2.20	1.67
63	0	56	24	2.32	1.67
64	0	64	16	1.96	1.50
65	0	72	8	1.76	1.40
55	8	72	0	1.52	1.45
45	16	64	0	1.70	1.40
36	24	56	0	1.49	1.42
28	32	48	0	1.40	1.30
21	40	40	0	1.60	1.15
15	48	32	0	1.60	1.10
10	56	24	0	1.35	1.20
6	64	16	0	1.20	1.20
3	72	8	0	1.32	1.20

In another experiment the wheat plants grew in solutions of glycocoll and methyl glycocoll, side by side at the same time, from February 22 to March 4. In this test only 11 different nutrient solutions were employed. One set of 11 cultures was used as a control; to each culture of the second set of 11 cultures 50 ppm. of glycocoll was added, and to the third set methyl glycocoll in the same amounts was added. The result of this test was very much the same as observed in the preceding experiments. The glycocoll produced increased growth in those solutions which contained no

nitrate and in those which had a small amount of nitrate, but only a very small increase in the solutions which contained larger amounts of mineral nitrate. The methyl glycocoll produced a stunted growth and a peculiar twisting and lateral growth of the top of the plant, as before noted. There was a 25 per cent decrease in growth from the control cultures.

Effect of calcium carbonate on the action of methyl glycocoll

An experiment was made to study the effect of methyl glycocoll under alkaline conditions. In this case 11 nutrient solutions were

FIG. 4.—Effect of glycocoll and methyl glycocoll on wheat plants in a nutrient solution containing 8 ppm. P_2O_5, 22 ppm. NH_3, and 48 ppm. K_2O; no. 1, nutrient solution; no. 2, same with glycocoll; no. 3, same with methyl glycocoll and calcium carbonate.

used, as in the experiment just recorded; one set of 11 was used as a control; another set contained glycocoll; and a third set methyl glycocoll with calcium carbonate. The physiological effect of the methyl glycocoll, in solutions with calcium carbonate, was the same as in the solutions already discussed. The effect of both the

glycocoll and methyl glycocoll is shown in fig. 4. The effect of the compounds was not materially different in the solutions of different composition. No. 1 is the control culture, no. 2 in addition to the nutrient salts contains 50 ppm. of glycocoll, and no. 3 contains 50 ppm. of methyl glycocoll with calcium carbonate. It will be observed that the plants in the glycocoll cultures are slightly larger than culture no. 1, the control. The characteristic effect of methyl glycocoll is shown in the third culture. The photograph shows the growth to be smaller and the stems twisted and leaves growing almost horizontally. The growth in the 11 cultures containing methyl glycocoll was 27 per cent less than in the control set, which shows that lime has not changed its physiological action.

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THE EFFECT OF SOME TRIVALENT AND TETRAVALENT KATIONS ON PERMEABILITY

W. J. V. OSTERHOUT

(WITH SEVEN FIGURES)

It has been shown[1] that there is a remarkable difference between monovalent and bivalent kations in their effects on permeability. None of the monovalent kations investigated (except H) are able to decrease permeability, while all of the bivalent kations investigated are able to do so to a marked degree. In view of this it becomes important to make similar investigations on the effects of trivalent and tetravalent kations.

It is desirable in these investigations to use salts which give neutral solutions, since, as has been previously shown,[2] both acid and alkali affect permeability. For this reason salts of lanthanum are especially useful; nitrates of yttrium and cerium were also employed, as they likewise give neutral solutions when used as here described. Some experiments were made with ferric sulphate and with aluminum salts, but these substances have the disadvantage of giving acid solutions.

The salts used were in all cases the purest obtainable and the distilled water was prepared with especial care.

A solution of $La_2(NO_3)_6 \cdot 12\ H_2O$ of the conductivity of sea water was made by dissolving 31.5 gm. in 297 cc. of distilled water. The concentration was about 0.126 M. A lot of tissue which had a resistance in sea water of 1350 ohms was transferred to the lanthanum solution. The resistance rose rapidly to a maximum of 2350 ohms after which it gradually fell. In a second experiment the resistance at the start was 880 ohms and the maximum resistance 1490 ohms. The results are shown in table I and fig. 1.

[1] OSTERHOUT, W. J. V., On the decrease of permeability due to certain bivalent kations. BOT. GAZ. **59**:315–330. *figs. 11.* 1915.

[2] ———, Extreme alterations of permeability without injury. BOT. GAZ. **59**:242–253. *figs. 4.* 1915.

At the beginning of the first experiment the resistance was 1350 ohms; from this we must substract the resistance of the apparatus (240 ohms) to get the resistance of the tissue itself or the net resistance. This was 1350−240=1110 ohms, and the net conductance 1÷1110=0.000901 mho. The net resistance at the maximum was 2350−240=2110 ohms, and the net conductance was 1÷2110=0.000474 mho. We may regard the permeability as equal to the conductivity, or for convenience we may, in such a case as this, regard it as equal to the conductance. The loss in permeability therefore was 0.000901−0.000474=0.000427 mho or 47.4 per cent.

TABLE I

ELECTRICAL RESISTANCE OF *Laminaria saccharina;* TWO EXPERIMENTS

Time in hours	In $La_2(NO_3)_6$ 0.126 M	In $CaCl_2$ 0.278 M	In sea water
0	1350	1300	1400
0.17	2080	1640	.....
0.33	2160	1730	.....
1	2350	1730	.....
1.83	2315	1640	.....
3.33	2030	1490	1400
10	1340	600	1370
18	880	400	1350
0	880	.....	890
0.5	1490	.....	.....
1	1360	.....	.....
2	1320	.....	.....
5.5	740	.....	.....
9.5	525	.....	890

All readings were taken at 18° C.

The net resistance at the beginning of the second experiment was 880−250=630 ohms, and the net conductance was 1÷630=0.00159 mho. The net resistance at the maximum was 1490−250=1240 ohms, and the net conductance was 1÷1240=0.00081 mho. The loss in permeability therefore was 0.00159−0.00081=0.00078 mho or 49.1 per cent. Similar results were obtained with La_2Cl_6. It will be seen that the rise in resistance is much greater in $La_2(NO)_6$ and La_2Cl_6 than in $CaCl_2$.

It has been pointed out[1] that the severest test of the ability of a salt to decrease permeability is to add the salt in solid form to the

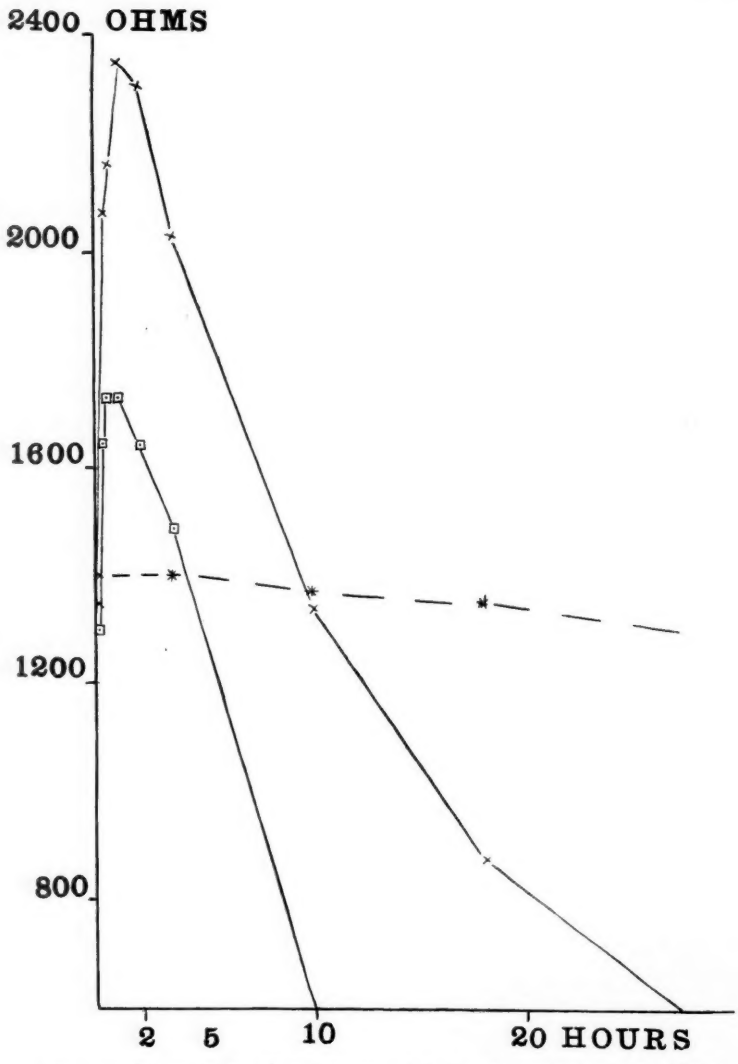


FIG. 1.—Curves of electrical resistance of *Laminaria saccharina* in $La_2(NO_3)_6$ 0.126 M (line with crosses), in $CaCl_2$ 0.278 M (line with squares), and in sea water (dotted line).

sea water. The results of such an experiment are shown in table II and fig. 2. To 275 cc. of sea water 5 gm. $La_2(NO_3)_6 \cdot 12\ H_2O$ were

TABLE II

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 275 cc. + $La_2(NO_3)_6 \cdot 12\ H_2O$ 5 gm. (=0.021 M)	In sea water
0	1090	1080
0.08	1210	1080
0.75	1210	.....
1.25	1210	.....
2	1190	.....
17	980	1030
22.5	935	1000

All readings were taken at 18° C.

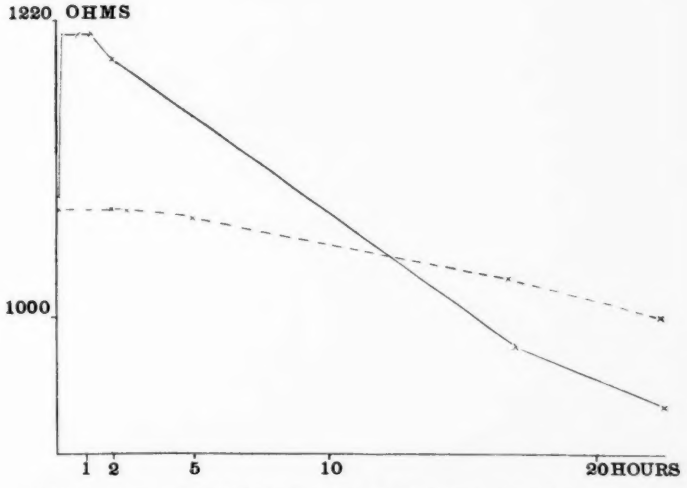


FIG. 2.—Curve of electrical resistance of *Laminaria saccharina* in sea water 275 cc.+$La_2(NO_3)_6 \cdot 12\ H_2O$ 5 gm. (=0.021 M) (unbroken line), and of a control in sea water (dotted line).

added, making the concentration 0.021 M. In this the resistance of a lot of tissue rose rapidly from 1090 to 1210 ohms, where it remained stationary for a time and then began to fall. In the course

of 17 hours it fell to 980 ohms, while that of the control fell 50 ohms in the same time. Dead tissue gave no rise in resistance.

TABLE III

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 300 cc.+ $Ce_2(NO_3)_6 \cdot 12\ H_2O$ 0.8 gm. = (0.003 M)	In sea water
0	840	810
0.16	860	.....
0.5	970	.....
1.5	990	.....
4	860	800
19	420	770

All readings were taken at 18° C.

The addition of the salt in solid form increases the conductivity of the solution. In order to produce a rise in resistance when added in this way, the action of the salt must be great enough to overcome the fall in the resistance of the solution which is contained in the apparatus and in the intercellular substance[3] of the tissue.

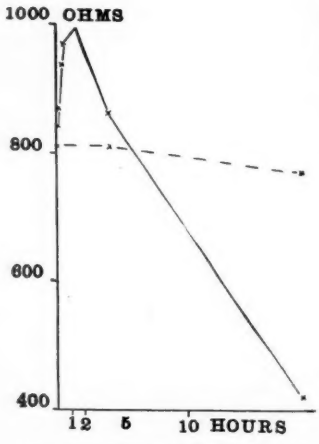


FIG. 3.—Curve of electrical resistance of *Laminaria saccharina* in sea water+$Ce_2(NO_3)_6 \cdot 12\ H_2O$ 0.8 gm. (=0.003 M) (unbroken line), and of a control in sea water (dotted line).

As has been pointed out,[1] such experiments furnish conclusive proof that the current passes through the protoplasm as well as through the intercellular substance.

In a previous paper the results of exposing the tissue alternately to sea water and to sea water+ $La_2(NO_3)_6$ were described in detail. The experiment shows that repeated exposure to sea water+ $La_2(NO_3)_6$ produces no injury.[4]

[3] The frond may be regarded as a mass of intercellular substance in which numerous masses of protoplasm (the cells) are imbedded.

[4] Science **36**:350. 1912.

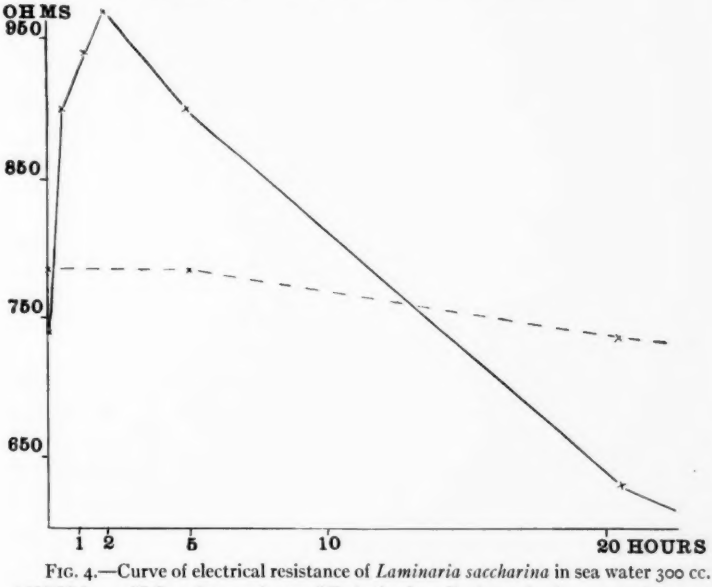
A lot of tissue which had in sea water a resistance of 840 ohms was placed in sea water to which $Ce_2(NO_3)_6 \cdot 12\ H_2O$ had been added (0.8 gm. to 300 cc. of sea water, making the concentration 0.003 M). In the course of 30 minutes the resistance rose to 970 ohms; during the next hour it continued to rise, reaching a maximum of 990 ohms, after which it slowly fell. The results are given in table III and fig. 3.

TABLE IV

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 300 cc. + $Y(NO_3)_3 \cdot 6\ H_2O$ 0.8 gm. (=0.007 M)	In sea water
0	740	770
0.5	900	.....
1.25	940	.....
2	970	.....
5	900	.....
20.5	630	735

All readings were taken at 18° C.



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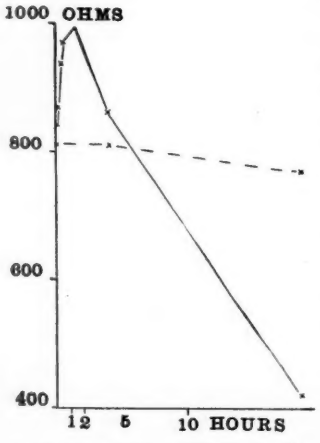

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TABLE IV

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 300 cc. + $Y(NO_3)_3 \cdot 6\ H_2O$ 0.8 gm. (=0.007 M)	In sea water
0	740	770
0.5	900	.....
1.25	940	.....
2	970	.....
5	900	.....
20.5	630	735

All readings were taken at 18° C.

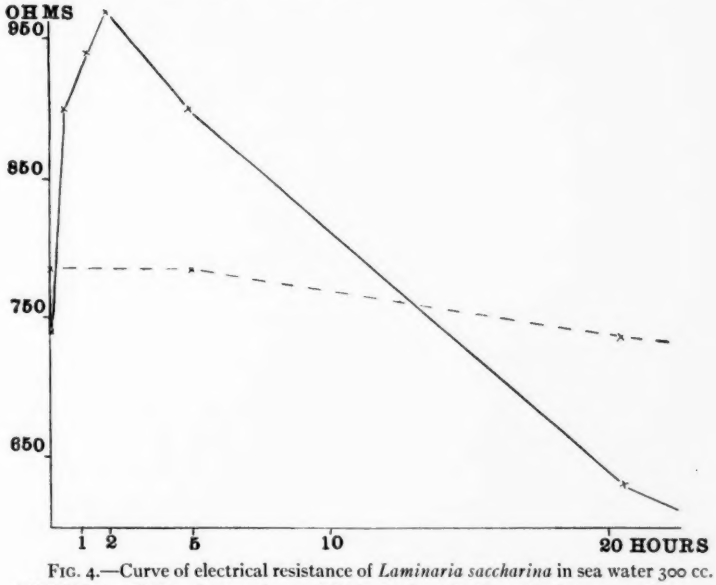

FIG. 4.—Curve of electrical resistance of *Laminaria saccharina* in sea water 300 cc. $+Y(NO_3)_3 \cdot 12\ H_2O$ 0.8 gm. = (0.007 M) (unbroken line), and of a control in sea water (dotted line).

A similar experiment was performed by adding 0.8 gm. $Y(NO_3)_3 \cdot 6\,H_2O$ to 300 cc. of sea water (=0.007 M) and placing a lot

TABLE V

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc.+ $Fe_2(SO_4)_3$ 1 gm. (=0.0025 M)	In sea water
0	750	730
0.5	810	.....
1.5	600	.....
17	500	670

All readings were taken at 18° C.

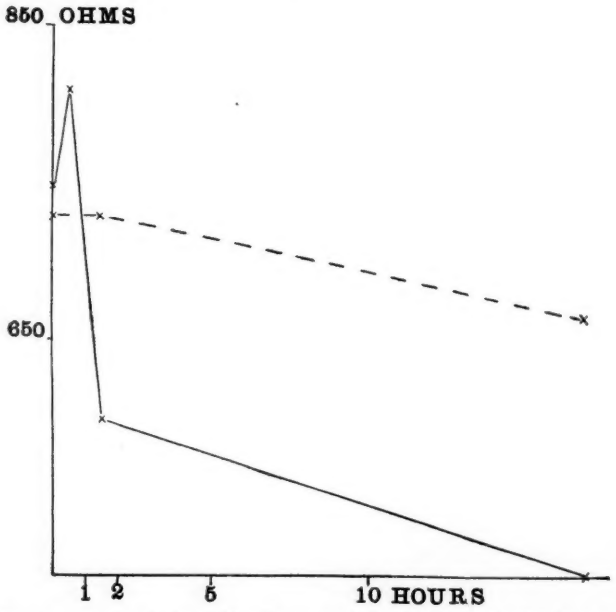

FIG. 5.—Curve of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+$Fe_2(SO_4)_3$ (=0.0025 M) (unbroken line), and of a control in sea water (dotted line).

of tissue in this mixture.[5] The tissue had in sea water a resistance of 740 ohms; after being transferred to sea water+$Y(NO_3)_3$ the

[5] It should be noted that if the dissociation were equal, a molecule of $Y_3(NO_3)_3$ would yield only half as many kations as a molecule of $La_2(NO_3)_6$ or of $Ce_2(NO_3)_6$.

resistance rose in 30 minutes to 900 ohms, and in the course of 2 hours reached 970 ohms; it then began to fall, and at the end of

TABLE VI

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 1000 cc. + $Al_2(SO_4)_3$ 18 H_2O 6.7 gm.	In sea water
0	800	840
0.5	1100	.....
0.75	1000	.....
1.25	950	.....
6.25	370	800

20.5 hours it was 630 ohms. The results are shown in table IV and fig. 4 (p. 469).

Another lot of tissue which had in sea water a resistance of 750 ohms was transferred to sea water 1000 cc.+ $Fe_2(SO_4)_3$ 1 gm. (=0.0025 M). The resistance rose in the course of 30 minutes to 810 ohms; at the end of 1.5 hours it had fallen to 600 ohms, and it continued to fall rapidly after this. The solution was acid to litmus, but the degree of acidity was not sufficient to account for the whole of the effect. The results are shown in table V and fig. 5.

Experiments were made with several salts of aluminum, including aluminum chloride, aluminum sulphate, ordinary alum, and chrome alum, which were added in solid form to sea water. All of them gave similar results. The solutions were acid, but the acidity was not great enough to account for the whole of the effect. The action of these salts is illustrated by table VI and fig. 6, which show the results obtained by adding 6.7 gm. $Al_2(SO_4)_3 \cdot 18\ H_2O$ to

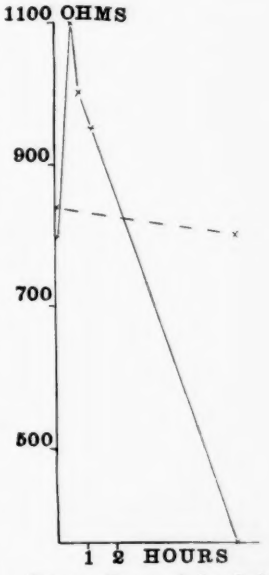

FIG. 6.—Curves of electrical resistance of *Laminaria saccharina* in sea water 1000 cc.+$Al_2(SO_4)_3 \cdot 18\ H_2O$ 6.7 gm. (=0.01 M) (unbroken line), and of a control in sea water (dotted line).

1000 cc. sea water (=0.01 M). It is evident that aluminum salts are very toxic.

As the result of plasmolytic investigations, FLURI[6] came to the conclusion that salts of aluminum (also of lanthanum and of

TABLE VII

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in hours	In sea water 300 cc. + Th $(NO_3)_4$ 0.4 H_2O 1 gm. (=0.006 M)	In sea water
0	690	720
0.5	750	.....
1	760	.....
2.5	680	.....
5	630	720
7.5	600	.....
29	540	660
40	510	640

All readings were taken at 18° C.

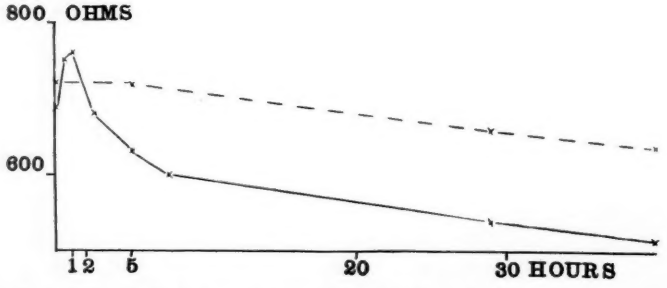

FIG. 7.—Curve of electrical resistance of *Laminaria saccharina* in sea water 300 cc. +Th $(NO_3)_4 \cdot 4\ H_2O$ 1 gm. (=0.006 M) (unbroken line), and of a control in sea water (dotted line).

yttrium) increase protoplasmic permeability. SZÜCS[7] made experiments on the taking up of dyes by the cell and concluded that salts of aluminum decreased the permeability. The experiments described show that both effects are produced. Which effect predominates depends both on the concentration of the salt (as is shown by experiments not mentioned here) and on the length of exposure to its action.

[6] Flora **99**:81. 1908.

[7] Sitzungsber. Wiener Akad. **119**: 1910.

In order to observe the effect of a tetravalent kation, 1 gm. $Th(NO_3)_4 \cdot 4\ H_2O$ was added to 300 cc. sea water, making the concentration 0.006 M. Tissue which had in sea water a resistance of 690 ohms was placed in this solution; the resistance rose in the course of half an hour to 750 ohms; at the end of one hour it was 760 ohms; after this it fell slowly, and at the end of 40 hours was 510 ohms. The results are shown in table VII and fig. 7.

Summary

All of the trivalent kations investigated (La, Ce, Y, Fe, Al) and the tetravalent kation Th are able to decrease permeability to a marked degree.

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Harvard University

PHYSIOLOGICAL ISOLATION OF TYPES IN THE GENUS XANTHIUM

CHARLES A. SHULL

(WITH SEVEN FIGURES)

While collecting *Xanthium* seeds for physiological studies, my attention has often been called to the heterogeneity of the *Xanthium* population in the field. Burs collected from several widely separated localities belong to the uncertain group listed under the name of *X. canadense* Miller in ROBINSON and FERNALD'S seventh edition of GRAY'S *Manual*.

In the annual report of the botanist of the Department of Agriculture for 1886, VASEY mentions *X. canadense* as the species troublesome in the west, while the principal eastern species is called *X. strumarium*. But it is now known that *X. strumarium* has never been introduced into America. As several types were often found growing intermingled in Kansas and Kentucky, the possibility of hybridization suggested itself.

Desiring seeds of uniform physiological character for certain investigations in which great accuracy was necessary, I collected burs from the three main types occurring in the fields about Lawrence, Kansas. In each case the seeds were chosen from a single plant of the type. It was thought that the various forms were possibly the result of promiscuous crossing of varieties or elementary species, and that a year or two of guarded pollination would be necessary to purify the strains so that physiological properties as well as morphological characters might be uniform. The use of pure bred material for physiological investigations has not yet been considered essential, but it may be very desirable, or even necessary, for certain kinds of work. Burs from the three types chosen are shown in figs. 1, 2, and 3. The original plants stood side by side on the northern edge of the Wakarusa floodplain about 0.5 kilometer south of Mount Oread. After being photographed, the burs were opened, and the seeds of the three types were found

to differ (fig. 4). The type with globose burs has much shorter seeds than the other two types, which are evidently more closely related. The seeds from type I have a dark brown testa, while those of type II have a dull greenish or grayish brown testa, and type III a yellowish brown testa.

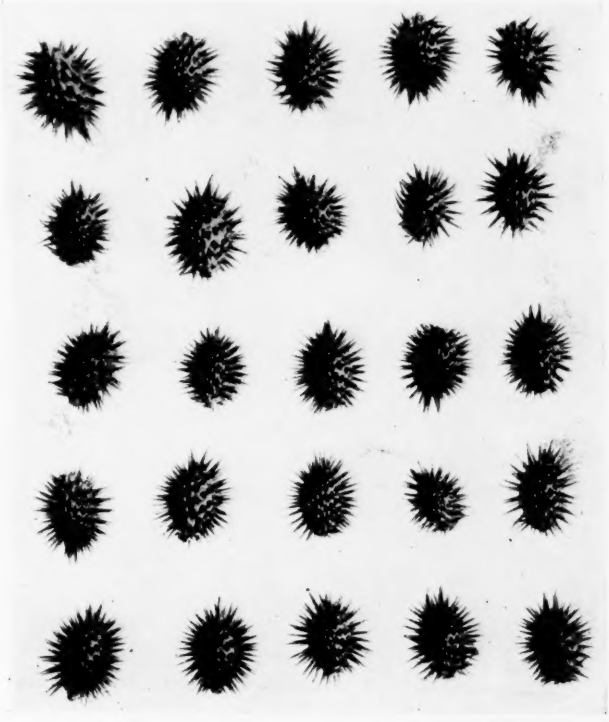

FIG. 1.—Type I, *Xanthium globosum* Shull, sp. nov.; natural size

The variation in size and weight of the seeds of the several types is under investigation. The average weight of 50 upper seeds of type I in the crop of 1913, an excessively dry year, is about 20 mg., of the lowers 27 mg. The corresponding average weights for type II are 28 mg. and 48 mg. respectively. The curves of variability in weight and length will probably show some overlapping in the

two types when a larger series has been measured, but it is certain to be slight.

Seedlings raised in the laboratory were transferred to the breeding grounds about June 1, 1913. Typical seedlings of types I and II are shown in fig. 5. The cotyledons of type I are somewhat

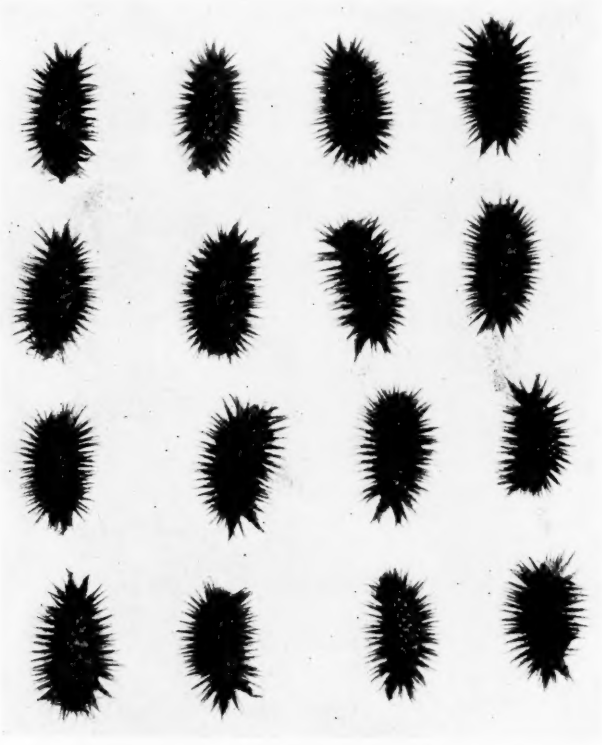

FIG. 2.—Type II, *Xanthium pennsylvanicum* Wallr.; natural size

shorter and broader than those of type II, which are long and strap-shaped; but the difference is not as great as would have been expected from the difference in the seeds.

The three types were planted within a day of each other; type I first, type II the following day, and type III last. As the plants

began to develop their characteristic mature leaves, a very surprising uniformity of the plants belonging to each type was observed. This result was wholly unexpected, as it was believed that hybridization could hardly have been avoided in nature. Type I has a dark green mesophyll with veins almost white, and a much

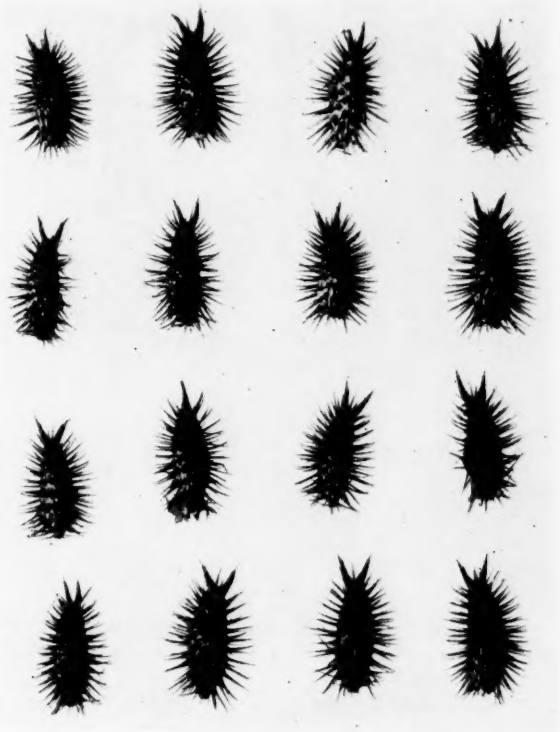

FIG. 3.—Type III, *Xanthium canadense* Mill.; natural size

crinkled surface; while type II has yellow green foliage, the leaf surfaces relatively plane. The foliage of type III resembles that of type I very closely, so that it is difficult to distinguish young plants (figs. 6 and 7 show the foliage differences of types I and II).

The most interesting difference between the two types is shown also in these figures. The photographs were taken at the same time, about September 1, 1913. Type II had shed all of its pollen and its burs were full grown. One shriveled cluster of staminate flowers can be seen above; while type I is just beginning to open its first staminate flowers, and its carpellate flowers are almost too small to be seen. Type III was intermediate, having about half

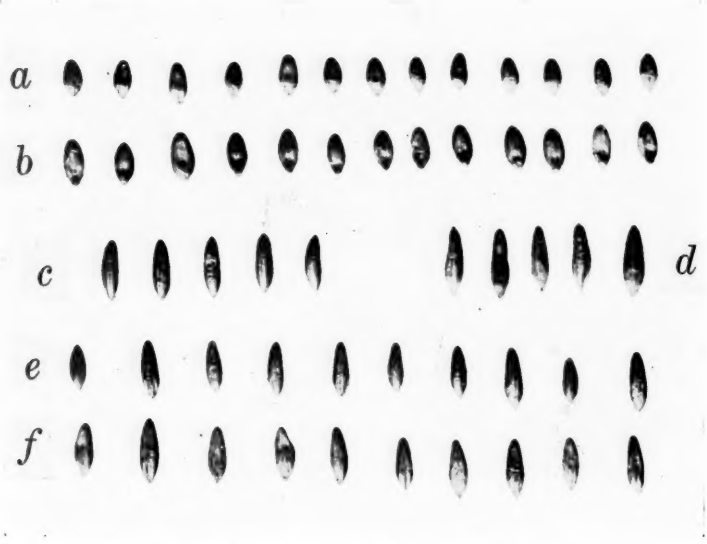

FIG. 4.—*a* and *b*, uppers and lowers of *X. globosum* Shull; *c* and *d*, uppers and lowers of *X. pennsylvanicum* Wallr.; *e* and *f*, uppers and lowers of *X. canadense* Mill.; all natural size.

grown burs at the time type I sheds its pollen; its flowers had no doubt been generally pollinated from plants of the same type. In this interesting condition we find the explanation of the remarkable uniformity exhibited by the offspring of plants taken at random from a heterogeneous population in the field. There is a physiological isolation that effectually prevents hybridization in the great majority of cases. The pollen of one variety has been shed long before the stigmas of the other are ready for the pollination processes.

During the summer of 1914, volunteer seedlings of types I and II were allowed to grow in the field under natural conditions, in competition with plants of the same types and other native weeds. The same differences in the burs and in the blooming times occurs under these circumstances, but the differences in the foliage loses some of its sharpness due to type I showing less crinkling of the mesophyll when crowded. There are wide differences

FIG. 5.—Seedlings of *Xanthium:* to the left, upper and lower of *X. pennsylvanicum* Wallr.; to the right, upper and lower of *X. globosum* Shull.

in the habit of the plants when crowded and when cultivated. The plants branch profusely when space permits, but are frequently unbranched or but slightly branched when closely crowded. Such differences will greatly alter the anatomical details of the plant structure, and tend to render such distinctions uncertain.[1]

One more difference between the three types remains to be noted. Type II develops considerable anthocyanin in the prickles

[1] The comparative anatomy of these three types of *Xanthium*, with *X. canadense* going under the name of *X. americanum*, has been described by NORA E. DALBEY (Kan. Univ. Sci. Bull. **9**:57–65. 1914).

and hairs that beset the burs, so that the rows of plants appear reddish brown long before the burs begin to dry. Type II develops much less anthocyanin, while pigment is entirely wanting in type I. When the burs have dried this difference is manifest in different shades of brown. The burs are lightest in color in type I, darkest in type II.

FIG. 6.—*X. pennsylvanicum* Wallr., at the same age as *X. globosum* Shull in fig. 7

Since the three types tend to remain distinct in the field, and since all of them show distinctive features while presenting no definite evidence of Mendelian segregation, they should be considered distinct species.

The genus *Xanthium* is in need of careful study, and possibly revision, on the basis of breeding tests. The principal difficulty in the genus from the taxonomic point of view is to interpret just what the old authors meant by *X. canadense* Mill. and *X. ameri-*

canum Walt. which was for a time called *X. strumarium* in the east, and which was redescribed by BRITTON in 1901 as *X. glabratum*. In the second edition of BRITTON and BROWN this species is now listed as *X. americanum* Walt. It seems clear from the descriptions that type III, with rather slender, oblong, essentially glabrous burs, should bear the name *X. canadense* Miller (Gard.

FIG. 7.—*X. globosum* Shull, at the same age as *X. pennsylvanicum* Wallr. in fig. 6

Dict. ed. 8. no. 2. 1768); with this species, *X. americanum* Walt. (1. Car. 231. 1788) and *X. glabratum* Britt. (BRITT. Manual 912. 1901) are synonymous.

Type II, with oblong pubescent fruit, agrees fairly well with the description of *X. pennsylvanicum* Wallr., and must on the basis

of breeding test be considered as a species distinct from *X. canadense* Miller.

The burs of type I do not resemble those of any of the 8 species now recognized in the territory east of the 100th meridian. This variety was first seen on the campus of the State University of Kentucky several years ago, where it formed a very small percentage of the cocklebur population. But here it was the most prominent variety in the field at the time the original selection was made in 1912. In addition, it is the most prolific type so far found. One specimen of average size bore 1864 burs, as compared to 75 on *X. speciosum*. It has been found to breed true, and since it is distinguished at once by its short smooth globose bur, I am naming it tentatively **Xanthium globosum.**

No study has yet been made of the geographical distribution and probable migrations of the several forms. The possibility suggests itself that *X. globosum* is a southern or southwestern form, developed in a region favorable to a long developmental period, and that *X. pennsylvanicum* is of more northern origin, with a correspondingly short period of growth. The two types may have met and mingled in the plains region without the possibility of a general hybridization because of the physiological isolation. An illustration of the behavior of a northern species may be cited in this connection. Seeds of *X. speciosum* Kearney obtained from South Dakota were planted about June 1, 1914, and had half grown burs by July 20, almost a full month before *X. pennsylvanicum* had developed its flowers, and six weeks before *X. globosum* was in bloom.

X. canadense Miller, as here interpreted, is in a number of ways intermediate between *X. globosum* and *X. pennsylvanicum*. It has an intermediate blooming period, intermediate sized burs and seeds, although the seeds stand much nearer to *X. pennsylvanicum* than to *X. globosum*, and intermediate production of anthocyanin. It has the oblong shape of *X. pennsylvanicum*, the glabrous character of the bur and the somewhat crinkly leaf of *X. globosum*. It is not yet known whether *X. canadense* can be produced by crossing *X. globosum* and *X. pennsylvanicum*, and selecting a pure form having the recombinations which it shows. While in general the

Xanthium population seems to be isolated according to species, there is always the possibility that the latest plants of one species may have the opportunity to cross with the earliest plants of a later blooming species. Such a cross between *X. globosum* and *X. pennsylvanicum* might possibly have given rise to *X. canadense*.

At a later time, when the range of variability of *X. globosum* has been carefully determined, the technical description of the species will be given. In the meantime the photographs will enable any one to identify the new species. There are probably a number of new species of *Xanthium* still undescribed in America, as would be indicated by the fact that half of the 8 species now recorded for the eastern half of the United States have been known less than sixteen years.

I am indebted to Mr. L. M. PEACE of the botany department of the University of Kansas for the excellent photographs of the types, and especially to Dr. J. M. GREENMAN, curator of the herbarium of the Missouri Botanical Garden, St. Louis, for examining the photographs and comparing the materials with specimens in the herbarium, and for information regarding the synonymy of *X. americanum* and *X. glabratum* with *X. canadense* Miller.

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THE ORIGIN AND DISTRIBUTION OF THE FAMILY MYRTACEAE

EDWARD W. BERRY

In the study of the lower eocene flora of the Mississippi embayment, a rather exhaustive compilation was undertaken to show the geological distribution of the genera present in that flora and the geographical distribution of the existing species in the families that were represented. This laborious work fully repaid the time involved, since many facts of general interest came to light and many highly suggestive, even if unproved, ideas emerged from the statistical tables.

A preliminary and somewhat tentative sketch of these matters was published,[1] and it is planned to elaborate the subject further in the final publication on the lower eocene flora under the auspices of the U.S. Geological Survey.

Among the various families that are represented in numerous fossil floras, none has excited greater interest among students of recent floras and geographical distribution than the family Myrtaceae, which in the genus *Eucalyptus* and its more immediate allies is so prominent an element in the present flora of Australia. What may be legitimately expected when fossil and recent floras shall have been studied in a sufficiently broad way, even with the present insufficient data of both recent and fossil geographical distribution, may be illustrated by the following brief sketch of what is known of the Myrtaceae.

The family Myrtaceae contains over 3100 existing species, separated by taxonomists into two subfamilies. The first of these, the Myrtoideae, with 32 genera and about 2400 existing species, comprises mostly tropical forms, of which over 75 per cent are confined to the Western Hemisphere. There are, however, over 200 species in Asia, one of which extends into Europe; about 75 species in Africa; about 200 species in Australia; and about 60 species in Oceanica. Nineteen of the genera are confined to

[1] Proc. Amer. Phil. Soc. **53**:129–250. 1914.

America, and these include the only monotypic genera in the subfamily (*Orthostemon* Berg, *Psidiopsis* Berg, and *Paivaea* Berg), as well as the large and greatly differentiated genera like *Myrcia* DC. with about 450 existing species. The two other large genera, *Myrtus* Linn. with about 178 existing species, and *Eugenia* Linn. with about 1300 existing species, are the only two genera found on all the continents (except Europe), and in these two genera America furnishes 135 species of *Myrtus* and about 850 species of *Eugenia*, or over 75 per cent in *Myrtus* and over 65 per cent in *Eugenia*.

The second subfamily, the Leptospermoideae, comprises the Leptospermae with 28 genera and about 700 existing species, and the Chamaelaucieae with 12 genera and about 165 existing species. Both of these tribes are even more strikingly Australian than the Myrtoideae are American. The Chamaelaucieae are entirely Australian and are mainly confined to Western Australia, and in accordance with their peculiar habitat, specialized characters, and restricted range, are probably of relatively recent origin. The Leptospermae have a single monotypic genus in Chile, and the distribution of the other members of this tribe suggests the probability that the South American genus should be placed in some other alliance, since with the exception of *Metrosideros* Banks, which is represented in Africa, and the genus *Baeckea* Linn., which reaches the Asiatic mainland, all of the genera are confined to Australia or to the surrounding islands southeast of Asia.

ANDREWS,[2] in a recent paper, has presented some interesting statistics of distribution and an ingenious theory of the history of the family. He considers that the original stock was arborescent or shrubby, with entire, simple, opposite, penni-veined leaves with dots and intramarginal acrodrome veins; with the calyx lobes and petals imbricate, probably in fives; flowers regular, solitary or in cymes; stamens indefinite, numerous, free, with versatile, 2-celled anthers; ovary inferior, with two or more cells; style simple; fruit inferior, crowned with persistent limb of calyx, indehiscent, succulent or fleshy (rarely dry); albumen none; cotyledons thick and fleshy, with a short radicle.

[2] ANDREWS, E. C., The development of the natural order Myrtaceae. Proc. Linn. Soc. N.S. Wales **38**:529–568. 1913.

From the character of cretaceous climates this or some other theoretical prototype flourished in a mesophytic environment. Among modern groups the nearest approach to this theoretical stock is furnished by the Myrtoideae which are fleshy fruited, most numerous in species, and widely spread in the equatorial regions of the world, with over 75 per cent, however, confined to America. The existing Myrtaceae with capsular fruits, representing the extreme of specialization in the family are Australian, while the Chamaelaucieae, standing in an intermediate position between the two preceding groups, are almost wholly confined to Western Australia.

These are the facts of modern distribution. Their interpretation may be various. ANDREWS (*op. cit.*), from a study of the present distribution, geologic climates, and the geological history of the Australian region, concludes that the Leptospermoideae originated from the Myrteae, and that the cretaceous forms were widespread, which latter was undoubtedly the case. He is convinced also that before the separation of Australia from the Asiatic mainland fleshy fruited forms found themselves in a region of warm moist climate, but relatively poor soil, and that it was this edaphic factor that was the principal stimulus to the differentiation of the Leptospermoideae, which with the exception of the genus *Metrosideros* Banks show adaptations to poor soil and temperate or dry climates, and this exception explains the relatively wide distribution of *Metrosideros* from Asia to the Fiji Islands. The *Eucalyptus* forms, according to the view of this student, were derived from *Metrosideros* after the separation of New Caledonia from Australia and the latter continent from Asia. To support this latter point ANDREWS is obliged to consider all of the cretaceous identifications of *Eucalyptus* and all of the tertiary identifications outside of Australia as equally misleading. With regard to the presence of *Eucalyptus* in North America I think this contention to be not unlikely, for although in accordance with paleobotanical usage I have identified numerous forms of *Eucalyptus* in the North American Upper Cretaceous, I have long thought that these leaves represented ancestral forms of *Eugenia* or *Myrcia*, but have hesitated suggesting any change based merely on personal opinion and also

from a consideration that such change in nomenclature is undesirable at the present time from the standpoint of stratigraphic paleobotany, which in this country, at least, is a most useful handmaid of geology.

The supposed cretaceous fruits of *Eucalyptus* have long since been shown to represent *Dammara*-like forms, and in my studies of the tertiary floras I have refrained from referring any of the numerous and unquestionable myrtaceous leaves to the genus *Eucalyptus*. Regarding the possible occurrence of *Eucalyptus* in the Tertiary of Europe, I am not sure that all of the identifications of HEER, UNGER, ETTINGSHAUSEN, and others are erroneous. Certain remains considered as *Eucalyptus* fruits by these authors seem very convincing from the published figures, and furthermore there is not the slightest doubt that the other great Australian alliance of the existing flora, the Proteaceae, was represented in both Europe and America during the Cretaceous and the Tertiary. There is an additional argument against the cretaceous radiation and the paleobotanical determination of *Eucalyptus* which is furnished by the great persistence in the modern forms of the peculiar juvenile, opposite, cordate, sessile, and horizontal leaves, a feature which must represent an ancestral character of long standing before the evolution of the falcate leaves of the genus with twisted leafstalks and other xerophytic features.[3]

I have dwelt at some length on this question because of its phylogenetic importance and the possible bearing of the American lower eocene floras on this point. In considering the morphology of the existing species, *Eugenia* has many claims to be considered the most primitive, although *Myrcia* is almost equally old and is certainly closely related to *Eugenia*. Among the numerous cretaceous fossils from North America now referred to *Eucalyptus* there is not a single one that does not exhibit characteristic features of *Eugenia* or *Myrcia*, especially of the latter, a fact greatly impressed on me in handling a large amount of recent material during my study of the American tertiary forms.

[3] See DEANE, H., Observations on the tertiary flora of Australia. Proc. Linn. Soc. N.S. Wales. **15**:463–475. 1900; and CAMBAGE, R. H., Development and distribution of the genus *Eucalyptus*. Presidential address, Jour. Proc. Roy. Soc. N.S. Wales, 1913.

In the lower eocene flora of the Mississippi embayment region there are six well marked species of *Myrcia* and four nearly equally well marked species of *Eugenia*, as well as a single species of *Calyptranthes*. The latter genus appears also to be represented in recent collections from the Oligocene of the Isthmus of Panama. Without pursuing the subject beyond the known facts, confessedly meager, and noting the presence in our lower eocene flora of numerous Combretaceae based upon leaves, flowers, and fruits, and a representative of the great tropical family Melastomaceae, largely American in the existing flora, both of which are families closely related morphologically to the Myrtaceae, it would seem that the known facts, as well as the law of probabilities, suggest America as the original home of the family. That in its early deployment it reached Europe, either by way of Asia or the North Atlantic plateau, early in the Upper Cretaceous, and became cosmopolitan before the close of the Cretaceous seems a most probable hypothesis. During the late Tertiary this ancestral stock, which largely coincided with the existing subfamily Myrtoideae, was forced to withdraw from temperate North America to the American tropics, where it had originated and to which it has since been so largely confined.

The types peculiar to the Australian region represent the relics of the cretaceous radiation with numerous new types evolved on that continent in the manner that ANDREWS has suggested, and at a comparatively recent date geologically. This is exactly the reverse of the hypothesis proposed by DEANE (*op. cit.*), but one that accords far better not only with the facts of geologic history, but also with those of existing distribution.

All of the American lower eocene forms are coastal types closely related to existing American species of similar habitat.

About 150 fossil forms have been referred to the family Myrtaceae, one-third at least having been described as species of *Eucalyptus*. At least half of these occur in the Cretaceous of all parts of the world, but particularly throughout the Northern Hemisphere. They are especially well represented in North America, and the possibility that they are ancestral forms of *Myrcia* or *Eugenia* has already been pointed out. A similar widespread distribution but less specific variation characterizes the

eocene forms that have been referred to *Eucalyptus*. The oligocene records are all European and the miocene records include both Europe and Asia.

The genus *Myrtus* L. has about 24 fossil species, all European, the majority being almost equally divided between the Oligocene and the Miocene. The oldest forms are early eocene, but the form-genus *Myrtophyllum* Heer has several upper cretaceous species in Europe, America, and Australia, as well as tertiary species in Europe, Asia, and South America.

The genus *Myrcia* DC., so well represented in the Lower Eocene (WILCOX) of our southern states, has species in the Middle Eocene (Claiborne) of the Mississippi embayment area, in the Oligocene (Vicksburg) of Louisiana, and in the European Oligocene. There are four species in the early Tertiary (Eocene or Oligocene) of Chile, one in the Tertiary of Ecuador, and one in the Pliocene of Brazil.

The genus *Eugenia* Linn., also prominent in our lower eocene flora, has its oldest known species in the Dakota sandstone (Upper Cretaceous) of the Rocky Mountain area. It is represented in Europe throughout the Tertiary from the Lower Eocene to the Pliocene, and is recorded by ENGELHARDT from the Tertiary of Ecuador.

The genus *Myrciaria* Berg, often included in *Eugenia*, has about 60 existing species, all American, and found in the area extending from the West Indies to Brazil and Peru. It is represented, according to ENGELHARDT, in the Tertiary of Ecuador.

The genus *Callistemon* R. Brown has been identified in both the Upper Cretaceous and the Tertiary of Europe, and no less than 25 fossil species have been referred to the genus *Callistemophyllum* Ettingshausen. These include upper cretaceous forms in both America and Europe; eocene or oligocene forms in Greenland; and numerous oligocene and miocene species in Europe and Australia.

The genus *Metrosideros* Banks, with an existing species in South Africa, another in the Sunda Islands, and 18 or 20 species in Australia or Polynesia, has 8 or 10 fossil species. The oldest, probably an erroneous identification, is recorded from the Atane

beds (Upper Cretaceous) of Greenland. There are 4 species in the Oligocene of Southern Europe and 2 species in the Miocene of that continent.

Leptospermum Forster, *Leptospermites* Saporta, and *Leptospermocarpum* Menzel have been identified from the Upper Cretaceous and Tertiary of Europe. *Tristania*-like fruits have been described as *Tristanites* by SAPORTA from the Lower Miocene of France and by KITSON from the Miocene of Australia. The genus *Psidium* Linn., with about 100 existing species in the West Indies and Mexico, is represented in Chile by an early tertiary species.

It will be seen from the foregoing enumeration that the facts of recent distribution lend support to the thesis of origin which is put forward, and this conclusion is not negatived but supported to a considerable extent by the confessedly meager evidence available in the geological record as at present known.

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GROWTH AND COLLOID HYDRATATION IN CACTI

ESMOND R. LONG

(WITH TWO FIGURES)

During the growing season of 1914 (March to August), attempts were made to correlate growth rates of cacti in solutions of varying reaction and concentration with the hydratation phenomena exhibited on placing cut pieces of the same species in similar solutions.

It was thought at first that the parallelism discovered by BOROWIKOW[1] in the growth rate and hydratation of *Helianthus annuus* might be expressive of a general property of plant colloids. This investigator found that acidity is a favoring factor in both the growing and the swelling rate of *Helianthus*, the amount of swelling and the amount of acceleration of growth being dependent upon the concentration of the acid, and differing with different acids, and that alkalies increase swelling and to a greater extent than acids, but do not appreciably affect growth. DACHNOWSKI[2] has obtained somewhat parallel results on the effect of acid with beans.

Experiments of a similar nature were performed in this laboratory upon *Opuntia Blakeana*, hydrochloric and malic acids (the latter being the acid existing in greatest concentration in the sap) being used as standards for the effect of acid, and sodium hydroxide as the standard for the effect of alkali. Growth rate and swelling were studied also in a nutrient solution made by diluting to 1100 cc. 100 cc. of the following solution: calcium nitrate 6 gm., potassium nitrate 1.5 gm., magnesium sulphate 1.5 gm., potassium monohydrogen phosphate 1.5 gm., sodium chloride 1.5 gm., and distilled water 600 cc.

For the experiments on growth, joints of *O. Blakeana* matured in 1913, bearing a small flower bud or new joint, were used, the old

[1] BOROWIKOW, G. A., Biochem. Zeitschr. **48**:230–246; and **50**:119–128. 1913.

[2] DACHNOWSKI, A., Amer. Jour. Bot. **1**:412–439. 1914.

joint being cut off smoothly at the base and placed in the appropriate solution, the latter being replaced by freshly prepared solution every few days. All the plants were suspended by means of wooden clamps in the various solutions to a uniform depth of 5 cm., and the battery jars containing the preparations were exposed to full sunlight in the laboratory court. While temperature and light conditions were thus not controlled, they were the same for all of the plants. The length of the new joint or bud was then measured at intervals of 3 or 4 days. In the swelling experiments, sections of standard size from healthy plants were cut with a cork borer, weighed, immersed in the different media, and kept in the dark in the laboratory constant-temperature room, the imbibed water being determined by weighing at 6-hour intervals during the following 24 hours. Inasmuch as there is considerable individual variation in swelling power in different joints from the same species, in all cases the experiments were conducted in such a way that all the joints used had equal representation in all the solutions of that experiment.

It developed immediately that for *Opuntia Blakeana*, with the concentrations of acid which it was practical to use with this plant, which has a concentration varying between N/10 and N/20 in its own sap, the observations recorded above for *Helianthus* do not hold. The results of these experiments are summed up in figs. 1 and 2 and table I, growth increment being expressed in millimeters,

TABLE I

GROWTH OF FLOWER BUDS OF *Opuntia Blakeana*, MARCH 25–APRIL 24

Medium	Total growth increment before flowering	Time in days
Distilled water	42.0 mm.	27
N/50 NaOH	40.5	30
N/50 malic acid	36.0	28
N/50 HCl	31.0	28

and swelling in percentage of the original weight. In nearly all cases the recorded figures represent the average for a number of plants, all of the hydratation experiments being carried out at least four times, and the majority of them twelve times.

The most striking fact brought out by the experiments is the inhibiting effect of acids upon both growth and hydratation, quite the opposite result from that obtained by BOROWIKOW with *Helianthus*. The discrepancy may be due in part to the stronger concentrations used in the experiment with *Opuntia Blakeana*,

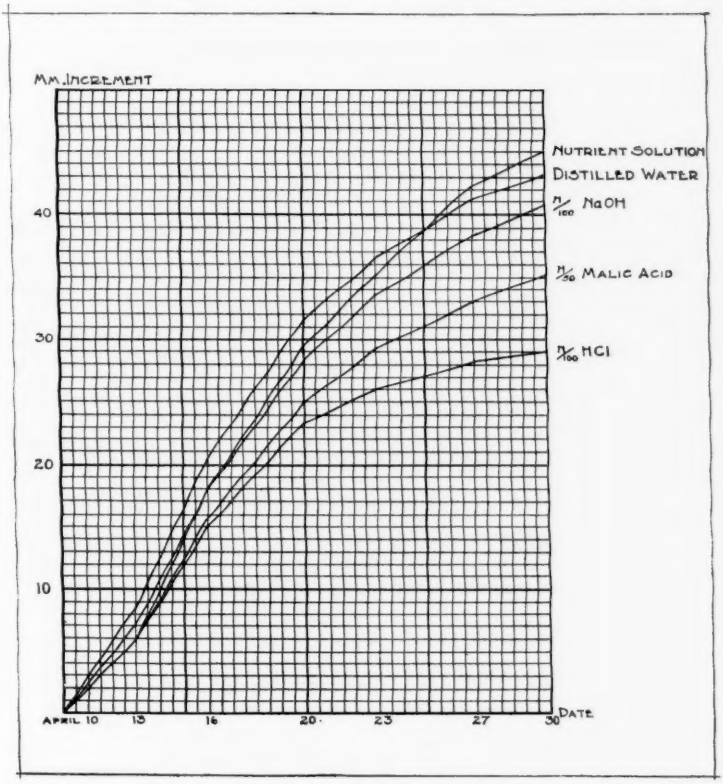

FIG. 1.—Growth of new joints of *Opuntia Blakeana*

BOROWIKOW'S concentrations ranging from N/100 to much lower. It might be argued that N/50 or even N/100 HCl could be toxic in other ways than in their effect upon water imbibition. The same could hardly be held, however, concerning N/50 malic acid, as this is considerably below the concentration of the same acid

in the cell sap. Nor is it likely that the lower swelling in acid solution is to be accounted for on the ground of osmotic pressure difference. The data in table II will give some idea of the rôle of osmotic pressure in water imbibition in this plant. The figures

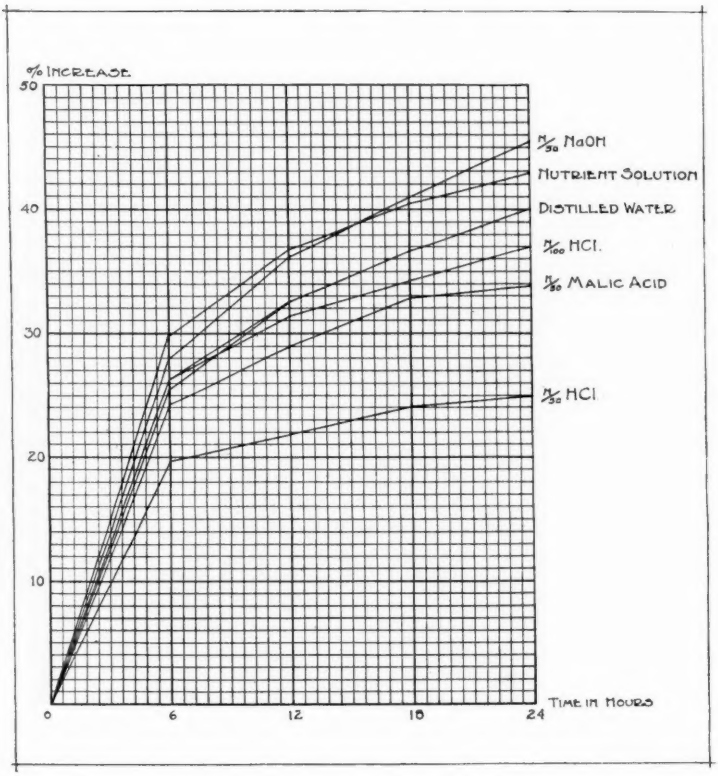

FIG. 2.—Hydratation of *Opuntia Blakeana*

given are for four pieces of *Opuntia Blakeana* in each solution, similar to those used in the hydratation experiments.

As may be seen, the swelling in distilled water and N/50 KNO_3 is practically the same, the slightly higher osmotic pressure of the latter being without measurable effect as regards increase in weight

due to imbibed water. It may be assumed that the osmotic pressure of the less ionized malic acid in N/50 concentration would also be without appreciable effect in the experiments in question.

TABLE II

Solution	Wt. at start	Wt. after 24 hrs.	Percentage increase
Distilled water	17.64 gm.	22.59 gm.	28.1
N/50 KNO_3	17.83	22.88	28.3
N/10 KNO_3	15.65	19.40	23.9
N KNO_3	16.75	13.77	−17.8

It seems probable, in view of these facts, that the lowered swelling in acid solution is the direct effect of the acid ion upon the colloids which take part in the swelling. The slower *growth* rate in acid solutions is significant in this connection.

The action of alkalies upon swelling was not so regular in its effect as that of acids. While a swelling of lower degree than that produced in distilled water was practically invariable with acids, the swelling produced in alkalies was sometimes greater and in other cases less than that taking place in distilled water, and no satisfying explanation can be given for this behavior. The varying acidity of the plant itself was perhaps a factor, by neutralizing to a greater or less degree the alkali of the penetrating medium. The curve represents the average of all cases examined.

It is interesting to observe that in nutrient solution, practically always a slightly increased swelling over that taking place in distilled water occurred, and furthermore that this was paralleled, as might be expected, by a greater growth rate in this medium. The mechanism of this action, which took place in a neutral medium, was not determined.

In general, growth and swelling in these experiments paralleled rather closely nutrient solution exerting an accelerating effect over that observed in distilled water, and hydrochloric and malic acids an inhibitory one, while the effect of sodium hydroxide was irregular.

While no attempt was made to separate and determine the colloid constituents of the plant to the presence of which the swelling

was due, it is evident that substances of much different physical nature from such colloids as gelatin and fibrin, which show a marked increase in swelling in acid solutions, are concerned. It should be noted that common cornstarch (boiled and dried) swells no more in N/50 and N/25 HCl than in distilled water, although the effect of NaOH of the same molecular strength in this direction is marked.

One important point left to be considered is the effect of the varying acidity of the plant sap upon the water-absorbing power of the plant. Dr. MACDOUGAL, to whom I am indebted for many suggestions in this work, has observed that the greatest growth rate of *Opuntia Blakeana* takes place in the day. In the daytime the acidity of the plant sap is on the decrease, the acid products of carbohydrate metabolism, after accumulating during the night, being destroyed by light and increased temperature during the day.[3] Can it be that this decrease has an effect upon the water-absorbing power and in consequence upon growth? The following experiment was carried out to test this. A joint of *Opuntia Blakeana* was cut at 5:00 A.M., 0.75 hr. before sunrise, the period of maximum acidity. Four disks were cut from it, and the holes plugged to prevent abnormal evaporation. The swelling of these in one hour in distilled water at 25° C. in diffuse light was determined immediately. The joint was exposed to the bright sunlight until

TABLE III

Time	Weight in grams	Increase in grams	Percentage increase
A.M.			
5:30	23.62		
6:30	25.25	1.63	6.9
P.M.			
4:30	22.25		
5:30	25.00	2.75	12.4

4:00 P.M., the period of minimum acidity, and then four disks were cut and their swelling determined in diffuse light for one hour, also at 25° C. The results are shown in table III.

[3] SPOEHR, H. A., Biochem. Zeitschr. **57**:95–111. 1913.

The swelling of the *less* acid sample is much the greater, and the difference is too great to be explained on the ground of increased osmotic pressure due to the day's transpiration. This result corresponds to those recorded above which had to do with pieces of the plant in acid and neutral solutions, and may be interpreted as possibly throwing light on the varying diurnal growth rate.

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CURRENT LITERATURE

NOTES FOR STUDENTS

Niter spots.—The occupation and cultivation of vast areas of semi-arid lands in Utah, Wyoming, and Colorado, which has been made possible by the development and extension of irrigation systems, has brought agricultural workers in these states face to face with many new problems. Possibly none of these has been more striking than that presented by the sudden appearance in the soil of nitrate accumulations in amounts sufficient to produce complete sterility over considerable areas which had previously been normally productive. Since the areas thus affected are widely scattered and embrace some of the most valuable agricultural lands of these states, the search for the causes of the condition has occupied the attention of a number of workers, and a very considerable literature, some of which borders upon the controversial in character, has resulted.

HEADDEN, who has been longest engaged in studies of the problem, was first to call attention to the extent and gravity of the condition, and to point out that most cases of sudden destruction of orchards, while popularly attributed to "black alkali" (sodium carbonate) from irrigation waters, were in reality due to nitrate accumulation. Contrary to HILGARD,[1] who attributed an increase in nitrates observed in certain irrigated soils of southern California to rapid transformation of organic matter previously accumulated in the soil, HEADDEN[2,3] has advanced the theory that fixation of atmospheric nitrogen by *Azotobacter*, with concurrent bacterial transformation of the resulting nitrogenous compounds into nitrates, is responsible for these accumulations. This author has shown that orchard trees, alfalfa, or other crops in the affected areas uniformly show characteristic injury quite unlike that produced by excessive irrigation or by sodium carbonate or other alkali salts, but identical with that produced by excessive use of nitrate fertilizers. He also attributes the rather widespread deterioration in yield, sugar content, and keeping qualities of sugar beets to accumulation of nitrates in the soil.[4]

[1] HILGARD, E. W., Soils. New York: Macmillan. 1911. pp. 68, 69.

[2] HEADDEN, WM. P., The fixation of nitrogen in some Colorado soils. Bull. Colorado Agric. Exper. Sta. no. 155. pp. 10–69. 1910.

[3] ———, Nitrates in the soil; an explanation of so-called "black alkali" or "brown spots." Bull. Colorado Agric. Exper. Sta. no. 160. pp. 1–8. 1910.

[4] HEADDEN, WM. P., Deterioration in the quality of sugar beets due to nitrates formed in the soil. Bull. Colorado Agric. Exper. Sta. no. 183. pp. 1–184. 1912.

HEADDEN's studies of the chemical composition of "niter spots" [5, 6, 7, 8] have shown that, in many cases, nitrates may be present in the first foot of the soil in amounts approximating 133 tons per acre-foot, while in many cases nitrate accumulation is progressing at rates varying between 2 and 22 tons annually per acre-foot. It is HEADDEN's contention that the quantities of nitrates found bear no constant ratio to the amounts of chlorides or carbonates present or to the total water-soluble salt content, and that the high nitrate content is confined to the first few inches of the soil, and he regards these facts, together with the extent of the affected areas and their dissimilar geological relations, as strong evidence against the theory that the condition can be due to seepage from nitrate-bearing rocks with subsequent deposition from surface evaporation.

SACKETT[9, 10] has attacked the problem from the bacteriological side and has presented evidence that *Azotobacter* is present in large numbers in irrigated soils, and that fixation of nitrogen occurs at exceptionally high rates in soils from "niter spots"; while ROBBINS[11] has shown that these soils are unusually rich both in species and numbers of Cyanophyceae, which may be considered as supplying carbohydrates available as a source of energy for *Azotobacter*. SACKETT further concludes from his studies of the ammonifying and nitrifying efficiency of Colorado soils[12, 13] that ammonification and nitrification proceed in them at rates considerably in excess of those found by workers in other parts of the United States. It must be said in criticism of SACKETT's work that, while his own ammonification cultures were in all cases continued for a period of seven days, he has compared the amounts of ammonia produced therein

[5] HEADDEN, WM. P., The fixation of nitrogen in some Colorado soils; a further study. Bull. Colorado Agric. Exper. Sta. no. 178. pp. 1–96. 1911.

[6] ———, The fixation of nitrogen in Colorado soils; the distribution of the nitrates and their relation to the alkalies. Bull. Colorado Agric. Exper. Sta. no. 186. pp. 1–47. 1913.

[7] ———, The excessive quantities of nitrates in certain Colorado soils. Jour. Indust. and Eng. Chemistry **6**:586–590. 1914.

[8] ———, Do Azotobacter nitrify? Science N.S. **40**:379–381. 1914.

[9] SACKETT, WALTER G., Bacteriological studies of the fixation of nitrogen in certain Colorado soils. Bull. Colorado Agric. Exper. Sta. no. 179. pp. 1–42. 1911.

[10] ———, Bakteriologische Untersuchungen über die Stickstoffbindung in gewissen Bodenarten von Colorado. Centralbl. Bakt. **34**[2]:81–115. 1912 (German translation of previously published paper just cited).

[11] ROBBINS, W. W., Algae in some Colorado soils. Bull. Colorado Agric. Exper. Sta. no. 184. pp. 24–36. 1912.

[12] SACKETT, WALTER G., The ammonifying efficiency of certain Colorado soils. Bull. Colorado Agric. Exper. Sta. no. 184. pp. 1–23. 1912.

[13] ———, The nitrifying efficiency of certain Colorado soils. Bull. Colorado Agric. Exper. Sta. no. 193. pp. 1–43. 1914.

directly with amounts found by other workers for similar cultures run for various shorter periods, as four or six days. Such results are in no wise comparable, since the initial rate of ammonification is normally low, but increases rapidly through the first six to ten days of incubation.

In the determinations of nitrifying efficiency, SACKETT employed soils obtained from various parts of the United States as checks upon soils from different portions of the state. Here the Colorado soils were collected under uniform conditions and with bacteriological precautions, while those from other states were taken by persons unfamiliar with bacteriological technique or with methods employed in soil sampling, and were subsequently shipped for long distances in widely varying types of containers. While the author deplores the failure to employ standard methods, he nevertheless draws rather sweeping conclusions as to the comparative ammonifying and nitrifying efficiencies of the two lots of soils, and asserts that the experiments demonstrate the presence in Colorado soils of a nitrifying flora distinct, either in species or in physiological efficiency, from that elsewhere found. It would seem to the reviewer that definite conclusions are not in order until concordant results have been obtained from repeated studies in which all possibility of variation in any significant factor has been eliminated. The necessity for caution is especially great here in view of the fact that the work of KELLERMAN and ALLEN[14] in Nevada, while showing the presence of large numbers of nitrifying and ammonifying bacteria, yields no indication of abnormally high rates of activity or of unusual activity of *Azotobacter;* while the more recent work of MCBETH and SMITH, to be presently discussed, is wholly confirmatory of the results obtained by KELLERMAN and ALLEN.

At the Utah Experiment Station, the problem has been attacked by STEWART and his co-workers. In the course of their very thorough studies of the production and movement of nitrates in the soils of the Greenville farm[15, 16, 17] STEWART and GREAVES reached the conclusion that bacterial action could not be responsible for the accumulation of any large quantities of nitrates at the surface of the soil, at least in irrigated areas, since nitrates are readily displaced into the deeper layers of the soil by the downward movement of irrigation water. In more than 30,000 determinations of nitric nitrogen,

[14] KELLERMAN, KARL F., and ALLEN, E. R., Bacteriological studies of the soils of the Truckee-Carson irrigation project. Bull. U.S. Dept. Agric., Bur. Pl. Industry. no. 211. pp. 1–36. 1911.

[15] STEWART, ROBERT, and GREAVES, J. E., A study of the production and movement of nitric nitrogen in an irrigated soil. Bull. Utah Agric. Exper. Sta. no. 106. pp. 67–96. 1909.

[16] ———, The movement of nitric nitrogen in soil and its relation to "nitrogen fixation." Bull. Utah Agric. Exper. Sta. no. 114. pp. 181–194. 1911.

[17] ———, The production and movement of nitric nitrogen in soil. Centralbl. Bakt. **34**[2]: 115–147. 1912.

extending over a period of eight years, these workers never found a nitric nitrogen content exceeding 300 pounds per acre for a total depth of ten feet. STEWART has also studied the rate at which nitrification occurs in this soil,[18] with results which indicate that the conditions rarely if ever permit this process to become intense, a conclusion in entire accord with the results of MCBETH and SMITH.[19]

The extensive and carefully controlled work carried on by these investigators for the past four years at the Greenville Experiment farm has shown that nitrification is practically confined in all cases to the first foot of the soil; that the application of irrigation water invariably diminishes the nitrifying power of the soil; that the water-content of non-irrigated soil, in a region having a well-distributed annual rainfall of 15.81 inches, is entirely too low throughout the summer months to permit of active nitrification; and that the nitrification process is markedly inhibited by the addition to the soil of small quantities of ammonium sulphate (170 ppm.). The scale on which these experiments were conducted, the attention given to the control of the experimental conditions, and the entire accordance of the data obtained for the four-year period, would apparently entitle the results of these authors to acceptance as conclusive.

STEWART and his associates have advanced the theory that abnormal accumulations of nitrates, wherever these may occur in the area which was submerged during later Cretaceous time, are due to transportation and deposition of leachings from the country rock, adducing proof that throughout Utah, Idaho, and Colorado, it is quite generally the case that the chlorine content of "niter spots" increases proportionately with the increase in nitrates, which they regard as conclusive evidence of a common origin of these elements. By recalculation of data presented by HEADDEN, these authors have shown that fairly definite ratios of increase between chlorine and nitrate hold for the Colorado nitrate areas, in one of which the supposed "fixation" of 621 pounds of nitrogen was accompanied by an increase of 236,883 pounds in the chlorine content of the first acre-foot! That any material activity on the part of nitrifying bacteria can occur in the presence of the amounts of chlorine shown by STEWART and GREAVES to be present in the soils studied by HEADDEN would seem impossible to the reviewer in view of the results of LIPMAN.[20] That *Azotobacter* can be responsible for an increase in soil nitrates which has been shown to occur at rates frequently exceeding 5000 pounds per acre-foot annually would seem equally impossible, since there would be required to supply the

[18] STEWART, ROBERT, The intensity of nitrification in arid soils. Centralbl. Bakt. **36**[2]:477–489. 1913.

[19] MCBETH, I. G., and SMITH, N. R., The influence of irrigation and crop production on soil nitrification. Centralbl. Bakt. **40**[2]:24–51. 1914.

[20] LIPMAN, C. B., Toxic effects of alkali salts on soils on soil bacteria. II. Nitrification. Centralbl. Bakt. **33**[2]:305–326. 1912.

necessary energy, under optimum conditions, at least 100 pounds of carbohydrate for every pound of nitrate formed.[21] Under no conceivable conditions could the algal flora of a soil supply any substantial portion of the 250 tons of dextrose needed for such fixation.

The most recent contribution to the subject is a study of the nitrate content of the country rock by STEWART and PETERSON.[22] While working primarily in Utah, these authors have collected and analyzed large numbers of sandstones, limestones, and shales from widely separated localities throughout Utah, Wyoming, and Colorado. These may be considered fairly representative of the country rocks occurring in the area covered by the cretaceous and tertiary seas. They find that while the Jurassic sandstones and shales are not characterized by an unusually high nitrate content, the cretaceous and tertiary sandstones everywhere contain nitrates far in excess of the quantities present in ordinary alkali-free soils, often to the amount of one to ten tons per acre-foot; while the tertiary shales have prevailingly an even higher nitrate content. Over very extensive, wholly barren areas of virgin "clay hill" soil there is present beneath the compact, impermeable surface clay a layer of ash-like material, two to six inches in thickness, bearing 0.15 to 0.20 per cent of sodium nitrate, an amount equal to 900 to 36,000 pounds per acre-foot. The authors estimate the total nitrate content of the Book Cliffs area in Utah and Colorado as being many times greater than that of the deposits of Chile, but have nowhere found concentrations of such extent and character as would permit them to be profitably worked, a situation resembling that found by FREE[23] in southern California. STEWART and PETERSON consider that the discovery that nitrate deposits are not confined to the shales, but are generally present in the country rock, and that their amounts are everywhere materially greater than has been hitherto supposed, constitutes conclusive proof that "niter spots" are accumulations resulting from leaching, and have no relation to bacterial activities in the soil. In view of the very large accumulation of evidence against the latter hypothesis and the conclusive character of the results obtained by STEWART and his co-workers, this conclusion would appear to be wholly justified.—JOSEPH S. CALDWELL.

Some temperature effects.—In discussing some of the phytogeographic effects of winter temperature, SHREVE[24] calls attention not only to the great lack of critical data, but more especially to the fundamental error, so prevalent

[21] MARSHALL, C. E., Microbiology. Philadelphia: Blakiston & Co. 1912. pp. 272–273.

[22] STEWART, ROBERT, and PETERSON, WILLIAM, The nitric nitrogen content of the country rock. Bull. Utah Agric. Exper. Sta. no. 134. pp. 420–465. 1914.

[23] FREE, E. E., Nitrate prospects in the Amargosa valley, near Tecopa, Cal. Circular U.S. Dept. Agric., Bur. Soils. no. 73. 1912.

[24] SHREVE, F., The rôle of winter temperature in determining the distribution of plants. Amer. Jour. Bot. 1:193–202. 1914.

in the past, of considering a degree in one part of the temperature scale the equivalent of a degree at any other part of the scale, as is done in the use of the annual mean temperature or even in totaling the degrees of temperature for the growing season. Although more attention has been given to the temperature phenomena of the growing season, he believes that the temperature phases of the frost season are perhaps of equal importance, especially in determining the distributional limits of some subtropical plants. He has already shown, as noted in this journal,[25] that the temperature conditions in mountains is often complicated by cold air drainage, but it would appear that in such situations winter temperatures are effective in determining the vertical limits of many species. Observations show that the number of consecutive hours of freezing temperature is the factor most closely corresponding in its distribution with the limitation of the species concerned. This would harmonize with SHREVE'S[26] experiments with the giant cactus, which show that the number of hours of exposure to temperature below freezing determines its death, without regard (within certain limits) to the absolute minimum reached. Thus *Cereus giganteus* is unable to resist freezing of over 19–22 hours duration, while other related Arizona species withstood periods up to 66 hours, and *Opuntia missouriensis* has been known to survive 375 consecutive hours of freezing temperature in Montana. The importance is thus emphasized of applying the exact quantitative methods of physiological work to plant geography in order to place its generalizations upon a secure logical basis.

In this connection it is interesting to note the method described by MCDOUGAL[27] of applying to the summation of temperature in hour-degree units for a given time a factor expressing the rate of growth of a particular species, in order to give the relative values of such temperature exposures.—GEO. D. FULLER.

Production of alcohol by higher plants.—MINENKOW,[28] investigating the question of alcohol production by higher plants fully aerated, and the influence of osmotic pressure and temperature on the process, finds that well aerated, sterile solutions of glucose (15.8 per cent), sodium sulphate (6–7.8 per cent), and di-potassium hydrogen phosphate (7.25 per cent) retard germination of *Vicia Faba* and favor alcoholic production so that the ratio of carbon dioxide to alcohol approached nearer the value observed for alcoholic fermentation than with seeds germinating in water. Growth was retarded by these

[25] BOT. GAZ. **55**:263. 1913.

[26] SHREVE, F., The influence of low temperature on the distribution of the giant cactus. Plant World **14**:136–146. 1911.

[27] MCDOUGAL, D. T., The auxo-thermal integration of climatic complexes. Amer. Jour. Bot. **1**:186–193. 1914.

[28] MINENKOW, A. R., Die alkoholische Gärung höherer Pflanzen. Biochem. Zeitschr. **66**:467–485. 1914.

solutions. With increasing concentrations of glucose (4–14 per cent), mannite (4–14 per cent), and Hellriegel's nutrient solution, there is a corresponding retardation of growth and increase of alcohol production. Low or high temperatures which retard growth also favor alcohol production, while at intermediate temperatures favoring growth alcohol production is decreased. The author therefore concludes that alcohol is produced by higher plants even under conditions of complete aeration, and correlates alcohol production with retardation of growth. It is immaterial whether the retardation of growth is brought about by unfavorable temperatures, high osmotic pressures, or other factors.—H. HASSELBRING.

Vegetation about Tucson, Arizona.—SHREVE[29] has compiled an excellent brief but comprehensive guide to the features of ecological interest in the vicinity of Tucson, Arizona. In addition to the better known desert and semi-desert areas immediately surrounding the city, he has included the more diversified conditions found in the adjacent Santa Catalina mountains. Starting with a desert formation at 900 meters, in which *Cereus giganteus*, *Opuntia* spp., *Echinocactus*, and *Fouquieria splendens* are conspicuous, the desert forms are found to disappear with increasing altitude, grasses and shrubs becoming more abundant, until at 1550 meters upon the north-facing slopes there is an open forest of such species as *Juniperus pachyphloea*, *Quercus oblongifolia*, *Q. Emoryi*, *Arctostaphylos pungens*, *Rhus trilobata*, and other woody forms. A further ascent of some 500 meters reveals forests of *Pinus arizonica* and smaller stands of other pines and oaks, with specimens of *Arbutus arizonica*. Finally, at 2350 meters this interesting succession finds its climax upon slopes forested with *Pseudotsuga*, *Abies concolor*, and *Pinus strobiformis*, with even more mesophytic forms along the water courses and in the undergrowth. A brief analysis is also presented of the factors which cause this diversity of vegetation. —GEO. D. FULLER.

Water reaction in a liverwort.—CANNON[30] reports experiments with a species of *Plagiochasma* found upon arid slopes of the Santa Catalina mountains, Arizona, at an altitude of 5000 feet, showing that the thalli are able to become air dry, involving the loss of over 70 per cent of their original weight; but upon being given water again they continued to grow without apparent injury. He has also demonstrated that these plants may endure such a desiccated condition for at least 25 days, and upon their restoration to moist conditions at once assume active growth. These experiments show clearly that this liverwort can withstand in nature conditions of extreme aridity.—GEO. D. FULLER.

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[29] SHREVE, FORREST, A guide to the salient physical and vegetational features of the vicinity of Tucson, Ariz. International Phytogeographic Excursion in Amer. pp. 11. 1913.

[30] CANNON, W. A., A note on the reversibility of the water reaction of a desert liverwort. Plant World **17**:261–265. 1914.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold face** type; synonyms in *italic*.

A

Aaronsohn, A., work of 78
Acacia 341
Achradelpha 337
Actinopelte 340
Adenocaulon bicolor, flower of 154
After-ripening, need of 431
Agave 336, 340
Alaska, sphagnum bogs of 262
Alcohol, production of 503
Allard, H. A., work of 80
Allen, E. R., work of 500
Alpine and subalpine vegetation of Lake Tahoe region 265
Alpine plant geography 64
Alway, F. J., work of 423
Amanitella 339
Amauriopsis 412
Amazonia 341
Ames, O., work of 336
Anderson, P. J., work of 163
Antarcticoxylon 421
Antevs, E., work of 264
Antholithes **pediloides** 332
Antholithus and Lepidopteris 264
Ant plants 73
Apogamy in Nephrodium 254
Araucaria brasiliensis, embryo of 1, 14; endosperm of 16; fertilization of 1, 5; morphology of 1; seed of 1, 16
Araucarineae 25
Arber, Agnes, work of 168
Archegonium of Sphagnum subsecundum 40
Ascomycetes 340
Aspen in reforestation 344
Aspergillus, toxic effects on 414
Aster 339
Aulacocarpus 340
Australia, flora of 337
Ayres, Jessie A. 154

B

Bachmann, E., work of 77
Baker, E. G., work of 77, 336
Bakeridesia 338
Bancroft, Nellie, work of 262
Banker, H. J., work of 336
Bartlett, H. H. 81; work of 183, 263
Bates, C. G., work of 76
Baur, E., "Experimentelle Vererbungslehre" 256
Beauverd, G., work of 336
Béguinot, A., work of 336
Belling, J., work of 160
Benedict, R. C., work of 77
Benzaitenia 342
Berger, A., work of 336
Berroa 336
Berry, E. W. 484; work of 422
Bessey, E. A., work of 259
Bews, J. W., work of 68
Bicknell, E. P., work of 336
Bidens **acuticaulis** 301; alausensis 310; **ambacensis** 309; andongensis 312; **arenicola** 309; **aurea** 313; aurea **leptophylla** 316; **Baumii** 309; **cinerea** 302; **elata** 312; **Elliotii** 309; floribunda 309; **grandis** 309; **Grantii** 309; **insecta** 309; **kilimandscharica** 309; **Kirkii** 309; odorata 304; **punctata** 302; **rufovenosa** 301; **ruwenzoriensis** 309; **Schweinfurthii** 309; studies in the genus 301; **Taylori** 309; tenella 311; **ugandensis** 309; **vincaefolia** 303
Bitter, G., work of 336
Blaauw, A. H., work of 67
Blake, Wm. P., work of 410
Blaringhem, L., work of 173, 177
Blepharanthera 337
Boeseken, J., work of 413, 415
Boldingh, I., "Flora of the West Indian Islands" 411
Bolivian plants 264
Botrychium, branching in 347
Bottomly, W. B., work of 419
Bovie, W. T. 149
Bower, F. O., work of 72
Brand, A., work of 336
Brannon, M. A., work of 410
Brenchley, Winifred E., work of 260
Briggs, L. J., work of 70
Britton, E. G., work of 336
Brockmann-Jerosch, H., work of 260

Brown, H. P. 197
Bryan, G. S. 40
Buddleia 338
Bulgariastrum 341
Burlingame, L. L. 1
Buscalioni, L., work of 336
Butters, F. K., work of 162

C

Cacti, growth and colloid hydratation in 491
Calcicoles 162
Caldwell, J. S. 498
California, vegetation of 80
Callitris 77
Calopeziza 341
Campanolea 337
Campbell, D. H., work of 260, 264; "Plant life and evolution" 158
Canadian zone 279
Cannon, W. A., work of 75, 76, 80, 504
Cardot, J., work of 336
Carex 339
Cattleya Mossiae 331
Cedrus, medullary rays of 387
Central American mosses 336
Cephalobembix 412
Chestnut disease 163
Chlamydoboea 341
Chrysler, M. A. 74, 387
Citropsis 341
Clark, G. H., and Malte, M. O. "Fodder and pasture plants" 258
Coal, origin of 69
Cockayne, L., work of 75
Cockerell, T. D. A. 331; work of 337
Coker, W. C., work of 75
Collins, G. N., work of 343
Coniferales 23
Connecticut, vegetation of 159
Contributors: Ayres, Jessie A. 154; Bartlett, H. H. 81; Berry, E. W. 484; Bovie, W. T. 149; Brown, H. P. 197; Bryan, G. S. 40; Burlingame, L. L. 1; Caldwell, J. S. 498; Chrysler, M. A. 74, 387; Cockerell, T. D. A. 331; Coulter, J. M. 60, 69, 72, 77, 78, 79, 80, 163, 164, 167, 168, 260, 262, 263, 334, 335, 412, 421; Cowles, H. C. 61, 66, 68, 70, 73, 74, 75, 76, 77, 78, 79, 80, 158, 159, 162, 165, 166, 167, 259, 260, 261, 262, 264; Crocker, W. 57, 59, 63, 67; DeVries, H. 169; East, E. M. 256; Emerson, R. A., 160, 165, 343; Farr, C. H. 136; Fromme, F. D. 164; Fuller, G. D. 60, 69, 71, 78, 79, 258, 262, 342, 344, 410, 420, 423, 424, 502, 504; Greenman, J. M. 61, 336, 411; Hasselbring, H. 412, 503; Hutchinson, A. H. 287; Land, W. J. G. 168, 258, 344, 397; Lipman, C. B. 402; Long, E. R. 491; Michell, Margaret R. 124; Osterhout, W. J. V. 242, 317, 464; Petry, L. C. 345; Reed, G. B. 409; Rigg, G. B. 258, 419, 422; Rose, D. H. 425; Shreiner, O. 445; Sharp, L. T. 402; Sherff, E. E. 301; Shull, C. A. 474; Skinner, J. J. 445; Smiley, F. J. 265; Spoehr, H. A. 366; Steil, W. N. 254; Vestal, A. G. 64
Cook, O. F., work of 337
Cordaitales 22
Cotton blossoms and bees 80
Couch, E. B., work of 79
Coulter, J. M. 60, 69, 72, 77, 78, 79, 80, 163, 164, 167, 168, 260, 262, 263, 334, 335, 412, 421
Cover glass, cleaning 401
Cowles, H. C. 61, 66, 68, 70, 73, 74, 75, 76, 77, 78, 80, 158, 159, 162, 165, 166, 167, 259, 260, 261, 262, 264; work of 422
Crataegus 340
Crocanthemum 336
Crocker, W. 57, 59, 63, 67
Crotalaria 77
Cubanthus 339
Cyathea 338
Cycadales 22
Cycadofilicales 20
Cyclocothis 337
Cylindrocarpon 342
Cyperaceae 339, 340
Cypripedium veganum 332
Cyrtandra 338
Cytherea bulbosa 331
Czapek, F., "Biochemie der Pflanzen" 59

D

Dachnowski, A., work of 262
Davis, B. M., work of 165, 173
Davis, J. J., work of 79
DeCandolle, C., work of 337
Defoliation and wood structure 261
Delf, E. M., work of 165
Dendrocousinia 339
Desert vegetation 74
DeVries, H. 169
Dichiliboea 341
Diedickea 337
Diedicke, H., work of 337
Douglass, A. E., work of 424
Douin, R., work of 344
Dümmer, R. A., work of 77
Dunn, S. T., work of 337
Dutch West Indian Islands, flora of 411

E

East, E. M. 256
Echinosporium 342
Egyptian cotton, mutation in 263
Elmer, A. D. E., work of 337
Elmerobryum 337
Embryo, of Araucaria brasiliensis 14; of Striga lutea 124
Emerson, R. A. 160, 165, 343
Endosperm of Araucaria brasiliensis 16
Engler, A., work of 337
Eremophyton 336
Escherich, K., work of 73
Euphorbiaceae 339
Evans, A. W., work of 168
Evaporation and plant succession 71
Everettia 339
Ewart, A. J., work of 337

F

Farr, C. H. 136
Fawcett, W., and Rendle, A. B., "Flora of Jamaica" 334
Fegatella, biology of 258
Ferdinandsen, C., and Winge, Ö., work of 264
Ferns, anatomy of some xerophilous 262; of Washington 258
Fiebrigia 337
Filicales, phylogeny of 72
Florida, notes from 259
Fodder and pasture plants 258
Food substances and growth 419
Forestation, sand hill 76
Forest, distribution and slope direction 422; service 78; growth studies in trees 197; mycorhiza of trees 66
Free, E. E., work of 261, 410, 502
Fritsch, K., work of 337
Fromme, F. D. 164
Frost, effect on seeds 435
Frye, T. C., and Jackson, Mabel M., "Ferns of Washington" 258
Frye, T. C., and Rigg, G. B., "Flora of the Northwest" 60
Fuchs, J., work of 66
Fuller, G. D. 60, 69, 71, 78, 79, 258, 262, 342, 344, 410, 420, 423, 424, 502, 504; work of 71

G

Gandoger, M., work of 337
Gametophyte of Picea canadensis 287
Gates, R. R., work of 175
Geerts, J. M., work of 177
Gelatin, imbedding in 400
Genetics, experimental 256
Geocarpon 339
Georgia, Ada E., "Manual of weeds" 257
Germination, delayed 425
Gleocarpus 340
Glycocoll, effect of 445
Gomphrena 341
Gongrospermum 340
Graff, P. W., work of 337
Grasses, leaf-sheath trichomes in 260
Greaves, J. E., work of 500
Greenman, J. M. 61, 336, 411; work of 377
Ground water, root characters and plant distribution 75
Growth, and colloid hydratation in cacti 491; and food substances 419
Guiana and Trinidad, vegetation of 260

H

Haberlandt, G., "Physiological plant anatomy" 60
Hall, H. M., work of 412
Hallier, H., work of 70, 337
Hamet, R., work of 338
Haraea 340
Hard-coatedness 425
Harper, A. G., work of 261
Harper, R. M., work of 259
Harshberger, J. W., work of 259
Hartsville, South Carolina, plant life of 75
Hasselbring, H. 412, 503
Hassler, E., work of 338
Hawkins, L. A., work of 417
Hayata, B., work of 338
Headden, W. P., work of 498, 499
Helminthostachys, branching in 347
Henslow, G., work of 167
Herzog, Th., work of 264
Heterostoma 341
Hevea 338
Hilgard, E. W., work of 498
Hitchcock, A. S., "Text book of grasses" 334
Hochreutiner, B. P. G., work of 338
Hopkins, L. S., work of 338
Hormisciopsis 341
Hosseus, C. C., work of 163
House, H. D., work of 338
Howe, C. D., work of 420
Howe, M. A., work of 168
Huber, J., work of 338
Hudsonian zone 283
Hutchinson, A. H. 287
Huxleya 337
Hydnaceae 336
Hydnodon 336
Hylocomiopsis 337
Hypericum 336

I

Iljin, W. S., work of 63
Imbedding in gelatin 400
Indonesian flora, origin and relationships of 70
Inflorescence, evolution of 72
Ireland, vegetation of Clare Island 166
Isodrepanium 336

J

Jaccard, P., work of 342
Jaenicke, A. J., work of 78
Jamaica, flora of 334
Janssonius, H. H., "Mikrographie des Holzes" 335
Javanese woods, micrography of 335
Jeffrey, E. C., work of 176
Jiménez, O., work of 338
Johnson, D. S., work of 163
Jost, L., "Pflanzenphysiologie" 57
Juel, H. O., work of 176
Jumelle, H., work of 338

K

Kaczmarek, R. M., work of 340
Kapteyn, J. C., work of 343
Kearney, T. H., work of 176, 263
Kellerman, Karl F., work of 500
Kellerman, M., work of 341
Kiesel, A., work of 415
Kinepetalum 337
Kirkwood, J. E., work of 342
Koehne, E., work of 338
Kraemer, H., "Applied and economic botany" 412
Kränzlin, F., work of 338
Kükenthal, G., work of 338
Kunkel, L. O., work of 416
Kurssanow, L., work of 164

L

Lacaitaea 336
Lacellina 340
Lake Tahoe region 265
Laminaria saccharina, permeability in 465
Land, W. J. G. 168, 258, 344, 397
Lasiothyrium 341
Lechmere, A. E., work of 339
Lembosina 341
Lepidocarpon 25
Lepidopteris and Antholithus 264
Lepidostrobus 168
Leptopharynx 412
Le Renard, A., work of 417
Lichens, antagonistic symbiosis in 79; relation to substratum 77
Life zones, Lake Tahoe region 276
Lindau, G., work of 339
Lipman, C. B. 402; work of 501
Liverwort, sporophyte of 344; water reaction in 504
Livingston, B. E., work of 416
Loesener, Th., work of 339
Long, E. R. 491
Lunell, J., work of 339

M

McBeth, I. G., work of 501
MacDougal, D. T., "Salton Sea" 410; work of 503
McDougall, W. B., work of 66
Mackenzie, K. K., work of 339
Macroglossum, morphology of 260
Madagascar, palms of 338
Maire, R., work of 339
Maize hybrids, first-generation 343
Malme, G. O., work of 339
Mamillaria 340
Marah 337
Marsh, A. S., work of 262
Marshall, C. E., work of 502
Mason, S. C., work of 339
Matthews, J. R., work of 424
Maybrook, Annie C., work of 258
Medullary rays of Cedrus 387
Melanographium 340
Melastomaceae 339
Menezesia 341
Merrill, E. D., work of 339
Merrill, G. K., work of 339
Methyl glycocoll, effect of 445
Mez, C., work of 339
Miadesmia 25
Michell, Margaret R. 124
Micropeltella 341
Miehe, H., work of 62, 73
Millspaugh, C. F., work of 339
Minenkow, A. R., work of 503
Mitrastemon 338
Monocotyledons, origin of 167
Monroe, C. E., work of 339
Morenoina 341
Morrison, A., work of 337
Muscatello, G., work of 336
Mutation, in Egyptian cotton 263; in Oenothera 81; in Oenothera biennis L. 169
Mycorhiza of forest trees 66
Myrtaceae, origin and distribution of 484
Myxomgriangium 341

N

Nash, G. V., work of 339
Natal, vegetation of 68

Nealchornea 338
Neosabicea 77
Nephrodium, apogamy in 254
Nesothamnus 412
New Guinea, flora of 335
New Zealand, evolutionary observations from 75
Nichols, G. E., work of 159
Nieuwland, J. A., work of 340
Niter spots 498
Nitrogen fixing, effect of moisture content on 402
Node, anatomy of 74, 167
North American flora 412
Nova Guinea 335

O

Ochoterena, I., work of 340
Oenothera 336; biennis L., mutation in 169; Lamarckiana a hybrid 172; mutation in 81; numismatica 86; pratincola, mutants of 86; reciprocal crosses of 165
Ohio, Polyporaceae of 80
Onagra, elementary species of 263
Ontario forest conditions 420
Ophioglossaceae, branching in 345; vascular anatomy of 345
Ophioglossum, branching in 346
Orchids 341
Osborn, T. G. B., work of 158
Ostenfeldiella 264
Osterhout, W. J. V. 242, 317, 464
Overholts, L. O., work of 80
Oxidases, distribution of 407
Oxygen, exclusion of 435

P

Paleobotanical notes 421
Paleozoic vegetation, ecological aspects of 263
Palestine, phytogeographic notes from 78
Palla, E., work of 340
Pappothrix 412
Paraffin, fixing of ribbons 398; solvent replaced by paraffin 397
Parish, S. B., work of 411
Parkin, J., work of 72
Paroxygraphis 341
Parry, C. C., work of 411
Pearson, G. A., work of 344
Peck, C. H., work of 340
Peirce, G. J., work of 410
Penicillium, toxic effects on 412
Peperomia 337; hispidula, morphology of 163
Peridium formation 164
Periolopsis 339
Peristomium 339
Permeability 317; effect of trivalent and tetravalent kations on 464; extreme alterations of 242
Perrier, H., work of 338
Peru, liverworts of 168; marine algae of 168
Peterson, W., work of 502
Petrakia 341
Petry, L. C. 345
Philippines, orchids of 336; plants of 337
Phoradendron 341
Photo-growth reaction 67
Picea canadensis, male gametophyte of 287
Pierce, R. G., work of 76
Pinus Strobus, growth studies in 197
Pittier, H., work of 340
Pityosporites 421
Plant distribution, root characters, and ground water 75
Plant succession and evaporation 71
Plasmodiophoraceae, new genus of 264
Polemoniaceae 336
Polygala 337
Polyporaceae of Ohio 80
Polystichum 341
Populus 340
Praeger, R. L., work of 166
Protoplasm, effects of Schumann rays on 149
Prunus 339
Psilosporina 337
Pygeum 338

Q

Quehl, L., work of 340

R

Radlkofer, L., work of 340
Rankin, W. H., work of 163
Reed, G. B. 409
Rees, B., work of 337
Reesia 337
Reforestation in aspen 344
Rehm, H., work of 340
Reid, C., "Submerged forests" 158
Rendle, A. B., and Fawcett, W., "Flora of Jamaica" 334
Respiratory activity and sunlight 366
Reviews: Baur's "Experimentelle Vererbungslehre" 256; Boldingh's "Flora of the West Indian Islands" 411; Campbell's "Plant life and evolution" 158; Clark's "Fodder and pasture plants" 258; Czapek's "Biochemie der Pflanzen" 59; Fawcett's "Flora of Jamaica" 334; Frye's "Ferns of

Washington" 258; Frye's "Flora of the Northwest" 60; Georgia's "Manual of weeds" 257; Haberlandt's "Physiological plant anatomy" 60; Hitchcock's "Text book of grasses" 334; Janssonius' "Mikrographie des Holzes" 335; Jost's "Pflanzenphysiologie" 57; Kraemer's "Applied and economic botany" 412; MacDougal's "Salton Sea" 410; Reid's "Submerged forests" 158; Rendle's "Flora of Jamaica" 334; Sargent's "Plantae Wilsonianae" 60
Rhizophora roots, branching of 80
Rhodopaxillus 339
Rhus 339
Rigg, G. B. 258, 419, 422; work of 262, 422
Ritter, G. C., work of 418
Robbins, W. W., work of 499
Rock, J. F., work of 340
Rolfe, R. A., work of 340
Root characters, ground water, and plant distribution 75
Rosa 339
Rose, D. H. 425
Rose, J. N., work of 340
Rosenstock, E., work of 340
Ross, H., work of 340
Rubiaceae 339, 342
Ruprechtiella 342
Rydberg, P. A., work or 64, 412

S

Saccardo, P. A., work of 340
Sackett, W. G., work of 499
Sand dune plants 79
Sand hill forestation 76
Sapindaceae 340
Sargent, C. S., "Plantae Wilsonianae" 60; work of 340
Saurauia 336
Schenck, H., work of 341
Schizochora 337
Schlechter, R., work of 341
Schreiner, O., 445; work of 419
Schumann rays, effects on protoplasm 149
Schuurmansiella 337
Sedum 338
Seedling anatomy 263
Seeds, of Araucaria brasiliensis 16; germination of 190
Selaginella, vegetative reproduction in 262
Selera 341
Semi-sterility, inheritance of 160
Senecio 337
Setchell, W. A., work of 263
Seward, A. C., work of 421
Shantz, H. L., work of 70
Sharp, L. T. 402
Sherff, E. E. 301
Shikotan, flora of 78
Shreve, F., work of 74, 503, 504
Shull, C. A. 474
Sinnott, E. W., work of 74, 167
Siphonostelma 337
Sirosphaera 341
Skinner, J. J. 445
Slope direction and forest distribution 422
Slosson, M., work of 341
Smiley, F. J. 265
Smith, N. R., work of 501
Smith, W. W., work of 341
Soil, available moisture of 423; studies 261
Solanum 336
Solms-Laubach, H., work of 177
Sorodiscus 342
Sphagnum bogs of Alaska 262
Sphagnum subsecundum, archegonium of 40
Spoehr, H. A. 366
Stapf, O., work of 341
Steil, W. N. 254
Stewart, R., work of 500, 501, 502
Stigmatomyces 340
Stigmatorhynchus 337
Stomatal activity 63
Stomps, Th. J., work of 180
Striga lutea, embryo sac and embryo of 124
Stuchlik, J., work of 341
Stuckertiella 336
Sumstine, D. R., work of 341
Sunlight and respiratory activity 366
Swingle, W. T., work of 341
Sydow, H. and P., work of 341
Sykes, G., work of 410
Symbiosis, chemistry of 76; hereditary 61
Symplocos 336
Szücs, J., work of 417

T

Takeda, H., work of 78
Talbotiella 336
Temperature effects 502
Thallochaete 341
Theissen, F., work of 341
Thiessen, R., work of 69
Thomas, E. N., work of 263
Torrend, C., work of 341
Tower, W. L., work of 179
Toxic effects 412
Transition zone 276
Transpiration in succulent plants 165
Traversoa 340

Treboux, O., work of 79
Tree growth 342, 424
Trelease, W., work of 341
Trichomanes 341
Trichymenia 412
Trotter, A., work of 340
Tucson, vegetation about 504
Turesson, G., work of 422
Turneraceae 342

U

Ulbrich, E., work of 341
Urban, I., work of 342

V

Van Leeuwen, W., work of 80
Vaupelia 336
Vestal, A. G. 64
Viola 338, 340
Vittaria 77
Von Faber, F. C., work of 61, 63

W

Washington, ferns of 258
Waterman, H. J., work of 413, 415
Water reaction in a liverwort 504
Water requirement of plants 69
Weaver, J. E., work of 71
Weeds, ecological study of 259; manual of 257
Wehmer, C., work of 414
Wernham, H. F., work of 77, 342
West Indian mosses 336
White, D., work of 69
White, J. H., work of 420
Wieler, A., work of 419
Wieland, G. R., work of 422
Williams, R. S., work of 336
Willis, J. C., work of 170
Wilson, M., work of 162
Winge, Ö., work of 342
Wisconsin, parasitic fungi of 79
Wollenweber, H. W., work of 342
Wood structure and defoliation 261
Woronichin, N., work of 342

X

Xanthium, inflorescences of 136; physiological isolation of types 474
Xyris 339

Y

Yendo, K., work of 342
Yucca 340

Z

Zellner, J., work of 76